GENETICS

A PERIODICAL RECORD OF INVESTIGATIONS
BEARING ON HEREDITY AND VARIATION

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HEREDITARY EFFECTS PRODUCED IN MAIZE BY RADIATIONS FROM THE BIKINI ATOMIC BOMB I. STUDIES ON SEEDLINGS AND POLLEN OF THE EXPOSED GENERATION[1]

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Received February 16, 1949

SEVERAL lots of maize seed were exposed to radiations from the first Bikini atomic bomb, the "Able" bomb of July 1, 1946. From these lots cultures were grown at the CALIFORNIA INSTITUTE OF TECHNOLOGY together with controls and a parallel series X-rayed by Dr. L. F. RANDOLPH. Cytological material was collected by DR. RANDOLPH for studies at CORNELL UNIVERSITY on visible chromosomal changes induced by the radiations (RANDOLPH, LONGLEY and LI 1948). Studies on hereditary effects have been carried on at the CALIFORNIA INSTITUTE OF TECHNOLOGY.

This report deals with the immediate generation of plants grown from the exposed seeds, and includes (a) observations on effects shown in the seedling stage and (b) observations and analysis of effects shown in the pollen.

SEEDLING TESTS

The first planting was made in the greenhouse July 28, 1946. Five lots of the F_1 hybrid seed, L289×1205, which had been exposed at Bikini, were included. These five lots had been recommended as probably coming within the range of exposure suitable for our studies. Untreated controls and lots subjected to 5,000, 10,000 15,000, and 20,000 r units X-ray were also included. Germination in all lots was about 98 percent, with the higher dosage lots showing no decrease below the controls or low dosage lots. A second planting of one lot of Bikini (lot A) and 15,000 r unit X-ray was made three days later. Further plantings were made at intervals until August 10.

In the X-ray comparison tests, seed treated with 10,000 r units or more gave a characteristic mottling and streaking of the early leaves of the seedlings. The first seedling leaves were heavily mottled instead of the uniform green of the controls. This is due to deleterious effects on individual cells in the leaf primordia. In the later leaves the spots became progressively more elongated, giving the leaves a finely streaked appearance. After about the fifth leaf, streaks were infrequent and the young plants took on a normal green color,

[1] Contribution from the Division of Biology, CALIFORNIA INSTITUTE OF TECHNOLOGY in cooperation with the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, UNITED STATES DEPARTMENT OF AGRICULTURE, and the NAVAL MEDICAL RESEARCH SECTION of Operation Crossroads.

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with only an occasional plant showing a pale or albino stripe or sector.

In a typical dormant corn embryo, five leaf primordia are visible as lateral outgrowths from the shoot axis, with the lower primordia having a larger number of cells. On any individual cell the radiation may have no detectable effect, or may have effects which prevent chlorophyll development, slow up cell divisions, cause early necrosis, or even prevent cell multiplication. From an already-formed multicellular leaf primordium, the resulting leaf is naturally a mosaic of tissues showing all of these effects. Where more cell divisions and growth must intervene as in the formation of the later leaves from the primary meristem, more rapid growth of the less injured cells and tissues will tend to eliminate cells and tissues which are handicapped by the more serious effects of radiation. Thus, there should be a gradual elimination of abnormal tissue. Abnormal stripes or sectors may survive if the abnormality does not markedly hinder the rate of cell division and development of the tissues.

In the X-ray series, seedlings from the 5,000 r treatment showed a small amount of mottling with little or no reduction in the size of the plants, while those from 10,000 r showed conspicuous mottling and averaged below normal in size. Seedlings from 15,000 r were very conspicuously mottled, and more reduced in size. A small percentage were weaklings which failed to survive. Most of the seedlings from 20,000 r appeared much like those from 15,000 r, but a much larger proportion (8 to 10 percent) were weaklings which did not survive. One lot of the Bikini series showed an intensity of mottling slightly below that of the 15,000 r X-ray treatment, with an absence of weaklings. The effect was much more uniform than in the X-ray series. There were no plants approaching normal and none which could be singled out as excessively affected. The reduction in vigor was more uniform and all plants recovered fairly evenly. The other four Bikini lots showed only slight traces of mottling.

POLLEN EXAMINATIONS ON THE EXPOSED GENERATION

Pollen examinations were made on plants of the first and second plantings. A portion of the central spike of each plant was collected, the fresh pollen was examined, and the occurrence of sectors of partial sterility was recorded. Sector size varied from a few spikelets to the greater portion of the spike, and in some spikes only partially sterile pollen was found. The limited area of the spike shedding pollen at the time of observation made it impossible to determine the size of sectors in most cases. The data are given only in terms of whether partially sterile pollen was observed on a given spike. No doubt many sectors of defective pollen were missed, so the total frequencies should be considerably higher than the observed frequencies.

For the X-rayed lots the observed frequencies were as follows:

	Plants examined	*With sectors of abnormal pollen*	*Percent*
5,000 r	176	52	29.5
10,000 r	170	90	52.9
15,000 r	532	335	63.0
20,000 r	135	95	70.4

In the 15,000 r lot, the first planting gave 72.3 percent and the second only 58.7 percent of spikes showing sectors. The order of planting was such as to place the second planting of 15,000 r at the end of the block. Usually collections and classifications of pollen were made in the order the cultures appeared in the field. It is possible that fatigue on the part of the observer may have contributed to the lower value in the second planting. The average value for 15,000 r should probably be slightly higher than the observed frequency of 63.0 percent.

For the lower dosages the frequency of observed sectors steps up rapidly with the dosage, but above 10,000 r units it levels off toward what may be a maximum of about 75 or 80 percent. Some leveling off is expected due to an increasing occurrence of multiple effects in the same cells. But a great part of the leveling off is no doubt due to the selective elimination of cells and tissues in which the effects are deleterious. The maximum may represent the average value beyond which the plants fail to survive the seedling stage.

The observed frequencies of sectors for the Bikini series follow, the five lots being designated A to E in the order of sector frequency.

	Plants examined	*With sectors of abnormal pollen*	*Percent*
Lot A	831	524	63.1
B	407	72	17.7
C	399	59	14.6
D	402	29	7.2
E	397	16	4.0

The pollen sector counts agree very closely with the seedling observations. Lot A, which seedling observation had placed as equivalent in biological effect to nearly 15,000 r, gave 63.1 percent of observed sectors as compared with 63.0 percent for the 15,000 r X-ray lot. First and second plantings gave nearly the same results, 64.4 and 61.8 percent, respectively. Lots B and C gave about half the frequency of sectors shown by the 5,000 r lot, and lots D and E were much lower. Unfortunately no lower dosages of X-ray were tested. As the dosage-frequency relationship below 5,000 r has not been investigated, we cannot safely extrapolate.

Partial pollen sterility is in nearly all cases due to chromosome alterations or rearrangements which give rise at meiosis to microspores deficient for some portion of a chromosome. Among these are translocations, inversions, and various types of deficiencies. A few pollen abnormalities due to other causes are expected, and likewise a few chromosomal alterations which do not visibly affect the pollen. Both should be low in frequency and should tend to cancel each other. Thus the frequency of partial pollen sterility can be taken as a direct measure of the frequency of chromosome alteration.

DATA ON SECTOR SIZE AND FREQUENCY

The preceding data give only the frequencies of plants in which sectors of partially sterile pollen occurred within the limited portion of the central spike shedding pollen on the day collected. In order to get an estimate of the size

TABLE 1

Size and frequency of partially sterile pollen sectors in terms of lateral tassel branches.

LATERAL BRANCHES PER TASSEL	PLANTS	NORMAL BRANCHES	PARTIALLY STERILE BRANCHES	½	1	1½	2	2½	3	3½	4	4½	5	5½	6	7	8	9	10	11	12	13	14	TOTAL BRANCHES
9	1	9	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	9
10	9	76½	13½	—	5	1	2	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	90
11	2	17½	4½	1	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	22
12	10	79½	40½	1	3	1	6	—	2	—	1	1	—	—	—	—	—	1	—	—	—	—	—	120
13	17	145½	75½	1	4	2	6	1	4	2	4	1	—	—	1	—	1	—	—	—	—	—	—	221
14	26	245	119	1	10	1	4	1	5	—	2	1	1	1	1	1	—	—	1	1	1	1	—	364
15	21	198½	116½	1	6	2	3	3	3	1	9	2	2	—	2	—	—	—	—	—	—	—	1	315
16	15	162½	77½	—	3	—	3	—	3	1	2	—	3	—	3	1	1	—	—	—	—	—	—	240
17	15	185½	69½	2	6	—	9	—	6	1	1	—	1	—	1	—	1	—	—	—	—	—	—	255
18	6	80	28	—	2	1	—	—	—	1	3	2	—	—	—	—	—	—	—	—	—	—	—	108
19	2	21	17	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	38
Total	124	1220½	561½	7	39	8	37	5	24	6	22	7	7	1	8	2	3	1	1	1	1	2	1	1782

and frequency of sectors, 124 whole tassels from Bikini lot A were examined, taking a sample of pollen from each lateral branch as well as from the central spike. Branches which showed both normal and partially sterile pollen were recorded as one-half of each. The number of lateral branches varied from 9 to 19 with a mean of 14.4. The counts for tassels having the same number of lateral branches have been grouped and the data are presented in table 1.

A total of 1,782 side branches were examined, of which 561½ or 31.5 percent had partially sterile pollen. These partially sterile branches were distributed in 183 sectors. The size of sectors varied from a portion of one branch to nearly the whole tassel, but the small sectors were most abundant. These frequencies may be combined and summarized as follows:

½ to 1	46	8	2
1½ to 2	45	9	1
3	29	10	1
4	28	11	1
5	14	12	1
6	9	13	2
7	2	14	1

While the mean size of observed sectors is approximately three branches, the median is two and one-half branches, that is, one half of the sectors are 2.5 branches or less in size, and an equal number 2.5 branches or larger.

To the 183 sectors recorded above, there should be added 10 sectors found only in the central spike. When these are included, the average number of partially sterile sectors becomes about 1.6 per plant. These were distributed on the plants as follows:

All normal	10
One sector	53
Two sectors	48
Three sectors	9
Four sectors	3
Five sectors	1[3]

These figures are based on sectors recognized as distinct in type of pollen. When several sectors of somewhat similar pollen type are present in the same tassel, it becomes virtually impossible to distinguish them. This difficulty is reflected in the abrupt drop in frequency of plants with more than two observed sectors. Except for the total of 31.5 percent of branches visibly abnormal, there is a systematic error running through all these figures. The abnormal tissue must actually be distributed in a larger number of sectors of smaller size than those observed. A reasonable estimate might well be an average frequency of between two and three abnormal sectors per plant, and an average size of sector about two branches.

[3] Only 3 types of pollen were present, with two small sectors of semisterile pollen on opposite sides of the tassel and similarly two small sectors with half of the pollen grains incompletely filled.

Assuming that the effects of the atomic radiations are immediate (not delayed to later cell generations) the above data indicate that, on the average, about seven or eight cells present in the growing tip of the irradiated dormant seed are represented in the reproductive cells of the tassel of the main stalk.

Data were also collected on the abnormal pollen sectors appearing in the central spikes of the same plants. Ten sectors were found which were not present in the lateral branches. Of the 183 sectors observed in the lateral branches, 113, or 61.7 percent, were represented in the central spikes. The other 70, or 38.3 percent, were not so represented. These sectors were distributed in the following size classes.

	1	2	3	4	5	6	Above 6
+	17	25	20	22	9	8	12
−	29	20	9	6	5	1	0

Sectors represented in the central spike have a mean of 3.7 lateral branches while those not so represented have a mean of 2.1.

Of the 124 plants observed, the central spikes of 98 showed some abnormal pollen, while 26 showed only normal. At the time of observation, the central spike had shed the entire length, and in some cases the middle portion had ceased shedding. Thus these observations are more inclusive than those made in comparing dosages. The 98 plants showing sectors of partial sterile pollen in the central spike had a total of 162 sectors affecting the lateral branches or an average of 1.7 sectors per plant, with a mean of 3.2 branches per sector. A total of 37 percent of the lateral branches were partially sterile. The 26 plants with only normal pollen in the central spike had only 21 sectors (0.8 sector per plant) with a mean of 1.7 branches per sector. A total of 10.1 percent of the branches were partially sterile. Thus the plants with one or more sectors of abnormal pollen in the central spike had about three and one-half times as many of the lateral branches abnormal as did the plants with wholly normal central spikes.

DISCUSSION

Any evaluation of the effects of atomic bomb or other radiations on maize seeds must be made upon the complex background of the developing embryo and plant. The impact of radiation is upon a partly grown although dormant plant, in which the growing tip and about five leaf primordia are many-celled structures. Before irradiation, all the cells of the embryonic plant are of the same genetic constitution. On exposure to the atomic bomb, each individual cell is exposed to the radiations, and each one may be expected to respond differently. Following the exposure each seed embryo may be looked upon as a collection of cells of differing genetic composition. Upon germination the seedling develops essentially as a colonial structure, being composed of a mosaic of sectors of which some or all may have been altered from their original constitution.

The best picture of the total effect of the atomic bomb radiations should be the first seedling leaf, which develops from the largest of the leaf primordia and by the fewest cell divisions. The second, third, fourth, and fifth leaves arise from successively smaller and fewer-celled leaf primordia. The percentage of chlorophyll-deficient or otherwise abnormal tissue is greatest in the first leaf, decreasing in each of the succeeding leaves. The later leaves arise from primordia formed from the apical meristem. These later leaves are mostly normal or almost normal in appearance.

It is probable that there is an equal effect of radiation on the undifferentiated cells of the apical meristem and of the already formed leaf primordia; and that the increasingly normal appearance is due to the more rapid growth and division of cells which have not been retarded by deleterious effects of the radiations. The competitive weeding out of handicapped cells should be most pronounced in the parts of the plant which are derived from the apical meristem. Thus the tassel sectors do not reflect the total effects of radiation but rather the composition of the relatively normal tissues which survive in the competition.

In the case of Bikini Lot A, the first seedling leaves were very strongly mottled, with probably more visibly abnormal than normal-appearing tissue. The tassels were nearly all entirely normal green in appearance, indicating the elimination during the growth of the plant of the majority of the apical meristem cells of the embryo. On an average, two or three sectors totaling 31.5 percent of the surviving tissue carried chromosome alterations. Thus in nearly one third of the relatively normal fraction of meristem cells ancestral to the tassel, the atomic radiations had produced chromosomal alterations of types which were not a handicap in the competitive growth which followed.

We can only speculate as to the genetic composition of the cells of the embryonic growing tip which were killed, or which were handicapped in competition. These must make up a large fraction of the meristem cells even at fairly low dosages. At dosages above 15,000 r the percentage becomes so great that frequently there are not enough vigorously growing cells left to enable the growing tip to function. As the dosage of radiation increases, there likewise takes place an increasingly rigorous selection for normality among the cells of the growing tip. Thus the pattern of genetic constitutions among survivors at different dosages should not be interpreted as simply a dosage-frequency relationship of the effect of radiation but also as the end result of selective competition.

SUMMARY

Plants from five lots of maize seed exposed to atomic bomb radiations were compared with lots exposed to 5,000 r, 10,000 r, 15,000 r and 20,000 r X-rays.

In seedling mottling, Bikini Lot A showed effects a trifle below the 15,000 r X-ray lot. The effects on the Bikini lot were more uniform.

The percentages of central spikes of the tassels showing one or more sectors of partially sterile pollen were

X-ray series		*Bikini series*	
5,000 r	29.5	Lot A	63.1
10,000 r	52.9	B	17.7
15,000 r	63.0	C	14.6
20,000 r	70.4	D	7.2
		E	4.0

Examination of 1,782 lateral branches from 124 tassels of Bikini Lot A showed 31.5 percent with partially sterile pollen. An average of 1.6 partially sterile sectors were recorded per plant. Owing to the difficulty of distinguishing different sectors in one tassel, the actual number was presumably larger. It is estimated that there were between two and three partially sterile sectors per plant with an average size of about two branches.

Data indicate that cell competition in the growth of the apical meristem eliminates the more affected cells, thus modifying the dosage frequency relations.

LITERATURE CITED

RANDOLPH, L. F., A. E. LONGLEY, and C. H. LI, 1948 Cytogenetic effects in corn exposed to Atomic Bomb ionizing radiation at Bikini. Science 108: 13–15.

IMMUNOGENETIC STUDIES OF DROSOPHILA MELANOGASTER
II. INTERACTION BETWEEN THE RB AND V LOCI IN PRODUCTION OF ANTIGENS[1]

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INTRODUCTION

THE discovery, at the turn of the century, of the human A-B blood groups, and the subsequent explanation of their heredity on the basis of triple alleles, opened a whole new field in genetics, one termed by IRWIN (1947 and previously) "Immunogenetics." Intensive search for such "genetic characters as yet only detectable by immunological reactions" has led to their discovery in such diverse animals as man and other primates, birds, rodents, cattle, and Protozoa. (The reader is referred to the following reviews: on immunogenetics, IRWIN 1947; on immunology, LANDSTEINER 1947; BOYD 1947.) With the exception of the antigens of Protozoa, where cytoplasmic inheritance introduces a novel situation (SONNEBORN 1947), most of these immunogenetic characters have been antigens of vertebrate erythrocytes.

From the genetic point of view, the most striking feature of this work has been the finding that, with but a few exceptions, there seems to be no interaction between genes involved in the production of antigens. This apparent one-to-one relationship between gene and antigen has led to the widespread suggestion that antigens stand in some sort of special relationship to genes, a relationship shared perhaps only with daughter genes and enzymes, in that they may be direct or primary products of their causative genes. If this be the case, immunogenetics should prove to be an important tool in the study of the nature of genic action and specificity (cf. WRIGHT 1945).

Whether or not this one-to-one relationship may be extended to all types of antigens is unknown. With only a few exceptions the relationship has been demonstrated for antigens of red blood cells. These substances, in the cases that have been analyzed, have proved to be haptens rather than complete antigens. Along with these immunochemical data, "the striking serological differences between the cells of individuals of the same species, the frequent occurrence of so-called heterogenetic antigens . . . , and the fact that blood cells of closely related species exhibit sometimes much greater differences than the respective serum proteins," led LANDSTEINER (1947, p. 76) to the conclusion that "there exist two systems of species specificity in the animal king-

[1] This article is a revised portion of a dissertation submitted to the faculty of the Division of Biological Sciences at the UNIVERSITY OF CHICAGO in candidacy for the degree of DOCTOR OF PHILOSOPHY. This investigation was aided by a grant from the WALLACE C. AND CLARA A. ABBOTT MEMORIAL FUND of the UNIVERSITY OF CHICAGO.

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dom, the specificity of proteins and that of cell haptens. The proteins, it would seem, undergo gradual variation in the course of evolution, while haptens are subject to sudden changes not linked by intermediary stages."

In the few cases where serum antigens have been subjected to immunogenetic analysis the one-to-one relationship seems to hold (CUMLEY and IRWIN 1942; CUMLEY, IRWIN, and COLE 1941, 1943; IRWIN and CUMLEY 1942). Unfortunately, the antigens in these cases have not yet been chemically identified, and while they are probably proteins this is by no means certain.

Certain other exceptional cases seem to argue against an invariable one-to-one relationship between gene and antigen. These are the "hybrid substances" that have been found in crosses between closely related species and within species (GORDON 1938; IRWIN 1932; IRWIN and COLE 1936; MCGIBBON 1944; SOKALOWSKAJA 1936; THOMSEN 1936). The explanation for the occurrence of these hybrid substances which first offers itself is the interaction in the hybrid of genes derived from the parents (IRWIN and CUMLEY 1945).

The existence of hybrid substances raises a number of questions which are crucial for the one-to-one hypothesis. Are the hybrid substances really exceptions, or are all or most antigens produced by gene interaction which has remained undetected due to the constancy of genetic background resulting from the inability to produce mutation on a large scale in vertebrates? If the hybrid substances are exceptions, wherein do the genes producing them differ in effect from those producing antigens without interaction?

It seems abundantly clear that the resolution of these problems will come only after the association of specific antigens with particular loci in an organism better adapted for genetic analysis than are the vertebrates.[3] A previous article (FOX 1949) reported the development of immunological methods capable of detecting antigenic differences between strains of *Drosophila melanogaster*. This article reports the initial results of the application of these methods to the problem of accomplishing the association mentioned above.

GENETIC MATERIALS AND METHODS

The most direct method available for the association of gene with antigen is that of inducing single visible gene-mutations in an isogenic stock and testing for ensuing antigenic change.

Derivation of isogenic stock.—Twenty separate attempts were made to derive an isogenic stock by means of the following series of crosses:

$$sc\ lz/ClB \times Cy\text{-}RNS/Pm^2;\ Payne\ Dfd\ ca/Sb$$

to give, in the F_1, females of the following genotype:

$$+/ClB;\ +/Cy\text{-}RNS;\ +/Payne\ Dfd\ ca.$$

[3] BURNET and FENNER (1948), in a review which appeared as this paper reached final stages of preparation, arrive at a number of conclusions similar to those reached here. In particular they urge "a combined biochemical, genetic, and immunological study of some convenient bisexual . . . organism" which would "eliminate the complications inherent in the use of vertebrate cellular antigens."

Such females, taken singly, were then backcrossed to their own fathers;

$+/ClB$; $+/Cy\text{-}RNS$; $+/Payne\ Dfd\ ca \times Cy\text{-}RNS/Pm^2$; $Payne\ Dfd\ ca/Sb$

yielding, among others, the four following types of offspring:

1. $+/Y$; $Cy\text{-}RNS/Pm^2$; $Payne\ Dfd\ ca/Sb$
2. $+/+$; $Cy\text{-}RNS/Pm^2$; $Payne\ Dfd\ ca/Sb$
3. $+/Y$; $Cy\text{-}RNS/+$; $Payne\ Dfd\ ca/+$
4. $+/+$; $Cy\text{-}RNS/+$; $Payne\ Dfd\ ca/+$

Flies of types 1 and 2 were mated singly *inter se*, giving the "Marker Stock" below, and flies of types 3 and 4 were mated singly in hopes of obtaining isogenic wild type flies among their offspring. In none of the twenty attempts, however, were wild type flies produced; each of the attempts to produce isogeniçity had rendered one or more lethal or semilethal genes homozygous. Instead, a balanced *Dfd* stock was obtained ("Isogenic Stock," below).

"Marker Stock"
$+/Y$; $Cy\text{-}RNS/Pm^2$; $Payne\ Dfd\ ca/Sb$
$+/+$; $Cy\text{-}RNS/Pm^2$; $Payne\ Dfd\ ca/Sb$

"Isogenic Stock"
$+/Y$; $+/+$; $Payne\ Dfd\ ca/l(3)F$
$+/+$; $+/+$; $Payne\ Dfd\ ca/l(3)F$

By means of this series of crosses all chromosomes in the isogenic stock except the 4th trace to a single source. Barring crossing over in the F_1 female (the likelihood of which is sharply reduced by the inhibitors present), and barring spontaneous mutations, the isogenic stock is actually isogenic in chromosomes 1 and 2, and the marker stock carries the same first chromosome as the former. Since the isogenic stock remains balanced, its genotype is as listed above.

The usefulness of the isogenic stock is not impaired by the fact that it is not wild type. Because of the balanced nature of the third chromosomes, and disregarding chromosomes 4 for the moment, all flies in the stock are genetically identical (again barring crossing over and spontaneous mutation). The presence of the dominant marker *Dfd*, as a matter of fact, has value as a check for contamination of the stock.

Lack of controi of the fourth chromosomes would seem to be the most serious source of heterogeneity in this stock. In the absence of suitable dominant markers and crossover inhibitors no attempt was made to render chromosome 4 isogenic. The results of this investigation seem not to have been influenced by this factor.

Derivation of mutant stocks.—Males of the marker stock were treated with 3000 r units of X-ray and mated to females from an attached-X yellow stock. Male progeny exhibiting "good" mutations were selected for further crosses designed to replace all rayed chromosomes except the single X-chromosome

bearing the mutant gene with unrayed chromosomes from the isogenic stock. Here again, no attempt was made to control chromosome 4.

The mutant males were mated to females from the marker stock (X′ indicates the X-chromosome bearing the mutant gene):

$$\left.\begin{array}{l} \text{X}'/\text{Y};\ \textit{Cy-RNS}/+;\ \textit{Sb}/+ \\ \qquad\text{or} \\ \text{X}'/\text{Y};\ \textit{Cy-RNS}/+;\ \textit{Payne Dfd ca}/+ \\ \qquad\text{or} \\ \text{X}'/\text{Y};\ \textit{Pm}^2/+;\ \textit{Sb}/+ \\ \qquad\text{or} \\ \text{X}'/\text{Y};\ \textit{Pm}^2/+;\ \textit{Payne Dfd ca}/+ \end{array}\right\} \times +/+;\ \textit{Cy-RNS}/\textit{Pm}^2 \quad \textit{Payne Dfd ca}/\textit{Sb}$$

to yield females of the following genotype among their offspring:

$$\text{X}'/+;\ \textit{Cy-RNS}/\textit{Pm}^2;\ \textit{Payne Dfd ca}/\textit{Sb}.$$

Each such female was then mated individually to one male from the isogenic stock, giving among their offspring males and females as indicated:

$$\text{X}'/+;\ \textit{Cy-RNS}/\textit{Pm}^2;\ \textit{Payne Dfd ca}/\textit{Sb} \times +/\text{Y};\ +/+;\ \textit{Payne Dfd ca}/\textit{l}(3)F$$

$$\text{X}'/\text{Y};\ \textit{Cy-RNS}/+;\ \textit{Sb}/\textit{l}(3)F$$

$$\text{X}'/\text{Y};\ \textit{Pm}^2/+;\ \textit{Sb}/\textit{l}(3)F$$

$$\text{X}'/+;\ \textit{Cy-RNS}/+;\ \textit{Payne Dfd ca}/\textit{Sb}.$$

These males and females were then mated singly *inter se*, yielding among their offspring

$$\text{X}'/\text{Y};\ +/+;\ \textit{Payne Dfd ca}/\textit{l}(3)F$$

and

$$\text{X}'/\text{X}';\ +/+;\ \textit{Payne Dfd ca}/\textit{l}(3)F.$$

Such males and females were mated individually to establish the mutant balanced stocks. The end result of this series of matings was, then, several stocks each of which may be considered to differ from the isogenic stock by a mutation at a single locus.

The mutations recovered by these methods were identified by means of tests for allelism. The two mutant stocks utilized in this investigation proved to be ruby and vermilion. Aceto-orcein smears of larval salivary glands disclosed no detectable X chromosomal aberrations in homozygotes, heterozygotes (with isogenic), and hemizygotes of each of the two mutant stocks.

A ruby-vermilion stock was derived from the two mutant stocks by mating ruby females with vermilion males; backcrossing the F_1 females to ruby males; mating the double mutant, crossover males to vermilion females; and extracting the double mutant stock by backcrossing females from the previous genera-

tion to double mutant males from the F_1 backcross. The resulting stock had the following genotype:

rb v/rb v; Payne Dfd ca/l(3)F

rb v/ Y; *Payne Dfd ca/l(3)F.*

This stock may be considered to differ from the isogenic stock by the mutations present individually in the two mutant stocks.

IMMUNOLOGICAL TECHNICS

The development of immunological technics suitable for an investigation such as this has been described in a previous paper (Fox 1949).

Preparation of antigens.—Flies of each stock, collected from mass cultures and starved as previously described, were lightly etherized, rapidly frozen while still alive, and lyophilized. For these experiments a special lyophile apparatus was available, and its use was substituted for the improvised apparatus used in the previous work. When desiccation was complete, the flies were suspended in 0.85 percent NaCl buffered at pH 7.4 by 0.005 M phosphate in the ratio of 1 gm of dried material to 50 ml of saline. Reduction in a Waring blender and grinding in a Potter-Elvejhem homogenizer followed, after which the suspensions were rapidly frozen and stored in a deep-freeze.

Production of antisera.—The complete antigenic preparations were used as injection antigens. Healthy rabbits of approximately 2,000 gm in weight were given four intraperitoneal injections of doubling dose on alternate days, the first injection being 1 ml of the appropriate antigenic suspension. Each rabbit therefore received 15 ml of antigen. Ten to twelve days after the last injection the rabbits were aseptically exsanguinated from the heart, following a 24 hour fast to avoid opalescent sera. The sera were separated, preserved with merthiolate 1:20,000, rapidly frozen, and stored in a deep-freeze.

Reaction technics.—Measurement of the titer of each antiserum with its homologous and heterologous antigens, and absorption of each antiserum by the various antigenic preparations, were accomplished simultaneously by use of the optimal-proportions method of Dean and Webb (1926).

The antigenic preparations were centrifuged; the clear supernates, separated from the packed particles, served as the "antigens" in all of the precipitin tests. Optimal-proportion determinations of each serum with its homologous antigen and heterologous antigens were performed. The tubes from the exact optimal-proportion determinations were incubated for two hours at 37.5°C and stored in the refrigerator for at least 24 hours. At the end of this period absorption was complete, and the precipitate was thrown down by centrifugation. The supernates were then tested for antibody content.

The method used in these latter tests was the modified precipitin-ring technic of Hanks (1935). The antigens, dissolved at optimal-proportion concentration in 5 percent gelatin, were layered into the bottoms of microprecipitin tubes and allowed to harden. The supernate from each of the optimal-proportion tubes was then carefully layered over the homologous and heterologous

antigens. The tubes were incubated in a water bath at 37.5°C for one hour, at end of which time they were examined for the formation of a precipitin-ring at the interface between antigen and antiserum. Storage at 6°C for 24 hours followed, at the end of which period the tubes were returned to the water bath for five minutes to remove non-specific clouding of the gelatin, and final readings were taken. The following controls were performed: antigenic controls, consisting of the antigens dissolved in 5 percent gelatin overlayered by buffered saline; antiserum controls, consisting of the supernates layered over 5 percent gelatin; normal controls, consisting of normal serum layered over the antigens in 5 percent gelatin. The precipitin-ring tests were read as follows: −, negative; t, trace; +, weak ring; ++, strong ring; +++, very strong ring. In both the optimal-proportions tests and precipitin-ring tests, 0.85 percent NaCl buffered at pH 7.4 by 0.005 M phosphate was used for all dilutions.

Early in this work it was found that the results were obscured by heavy, non-specific precipitation of the antigenic preparations. The precipitate so obtained was found to be ether-soluble, and was therefore probably lipidal in nature. This difficulty had not been encountered in the previous work, and probably occurred here because of better preservation of organic material by the improved lyophile apparatus. The non-specific material was removed from the antigens by means of three successive extractions, in a separatory funnel, with cold ethyl ether. As a check against alteration of antigenic structure during this extraction, each precipitin-ring test was repeated using antigens extracted at different times. No evidence of alteration was encountered.

It should be noted that the supernates from a particular optimal-proportion determination were tested with all antigens simultaneously. This procedure reduced the possibility of subjective error, since the strength of reaction of the supernates with the homologous antigen could be directly compared with the strength of their reactions with the heterologous antigens.

RESULTS

Detailed results are given only for the optimal-proportions tests and for the analysis of isogenic. The data upon which the analysis of the other stocks are based have been deposited in the editorial office of GENETICS.

Optimal-proportions tests.—Table 1 gives the protocol used and the results obtained in a typical exact optimal-proportions test. The same protocol was employed in all such tests, except that in each case the initial antigen dilution was adjusted according to the results of the rough determination.

Table 2 gives the results of the optimal-proportions tests. For each antiserum the result with the homologous antigen is given first. The results for the anti-isogenic, anti-vermilion, and anti-ruby-vermilion sera indicate significantly higher titers for the tests with the homologous antigens. The anti-ruby serum gave no such results—the titers for all three antigens being substantially the same. There is a remarkable agreement between these differences and the results of the precipitin-ring tests reported below. In those tests, the same antisera demonstrate an antigenic fraction possessed alone by isogenic and a

TABLE 1

Protocol for exact optimal-proportion tests.

Serum: S-13, Anti-isogenic
Dilution—1:2
Antigen: D-3-1, vermilion
Initial dilution—1:50

Incubation temperature: 37.5°C

Optimal proportion:* 40

TUBE	1	2	3	4	5	6	7	8
Saline (ml)	—	0.1	0.15	0.2	0.23	0.25	0.27	0.29
Antigen (ml)	0.4	0.3	0.25	0.2	0.17	0.15	0.13	0.11
Antigen dilution	1:50	1:67	1:80	1:100	1:118	1:133	1:154	1:182
Antiserum (ml)	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Time to pp't. (Min)	20	19.75	18.25	19.5	21	23	24.5	25.75

$$* \text{ Optimal-proportion} = \frac{\text{Dilution of antigen}}{\text{Dilution of antiserum}} = \frac{80}{2}$$

fraction possessed in common by ruby-vermilion and vermilion, but fail to demonstrate a fraction specific to ruby. The differences between optimal-proportions may, then, be due to these antigenic differences.

TABLE 2

Optimal-proportions.

ANTISERUM	ANTIGEN	OPTIMAL-PROPORTION
S-12 (Anti-isogenic)	D-1-1 (isogenic)	12.3
	D-2-1 (ruby)	50
	D-3-1 (vermilion)	40
S-14 (Anti-ruby)	D-2-2 (ruby)	29.4
	D-1-2 (isogenic)	21.4
	D-3-2 (vermilion)	29.4
S-15 (Anti-vermilion)	D-3-3 (vermilion)	4.2
	D-1-3 (isogenic)	12.3
	D-2-3 (ruby)	8
S-20 (Anti-ruby-vermilion)	D-4a-1 (ruby-vermilion)	6.2
	D-1a-1 (isogenic)	37.6
	D-2a-2 (ruby)	42.72
	D-3a-1 (vermilion)	2.7–3.1

Antigenic analysis of isogenic.—Tables 3, 4, and 5 give the results which allow analysis of the antigenic structure of isogenic. Antigenic controls, antiserum controls, and normal controls were all negative. Absorption of the anti-isogenic serum by the homologous antigen removed all antibodies, indicating specificity of the serum.

The results summarized in tables 3 and 4 indicate that upon absorption of the anti-isogenic serum with increasing amounts of either ruby or vermilion antigens a point is reached where all antibodies to antigens possessed in common by isogenic and the two mutants are removed, leaving antibodies to antigens specific for isogenic. This point is reached almost simultaneously for both mutants, regardless of which is used for absorption. This finding can only mean that neither mutant possessed, to the first order, more in common with isogenic than does the other, and that isogenic possesses an antigenic fraction lacking in both of the mutants.

TABLE 3

Analysis of isogenic with respect to ruby.

Serum: S-13, Anti-isogenic Absorbing Antigen: D-2-1, ruby

Precipitin-Ring Tests—Hanks Technic. 24 hours readings.

ANTIGEN	TUBE							
	1	2	3	4	5	6	7	8
D-1-1, isogenic (1:12 in gel.)	++	++	++	++	+++	+++	+++	+++
D-2-1, ruby (1:50 in gel.)	−	−	−	t	+	+	+	+
D-3-1, vermilion (1:40 in gel.)	−	t	t	+	+	+	++	++
D-1-1, isogenic (1:12 in gel.)	++	++	++	++	++	+++	+++	+++
D-2-1, ruby (1:50 in gel.)	−	−	−	−	t	+	+	+
D-3-1, vermilion (1:40 in gel.)	−	−	t	t	+	+	+	++
D-1-2, isogenic (1:12 in gel.)	++	++	++	++	++	+++	+++	+++
D-2-2, ruby (1:50 in gel.)	−	−	−	t	t	+	+	+
D-3-2, vermilion (1:40 in gel.)	−	t	t	+	+	+	+	++
D-1-2, isogenic (1:12 in gel.)	++	++	++	++	++	+++	+++	+++
D-2-2, ruby (1:50 in gel.)	−	−	−	t	+	+	+	++
D-3-2, vermilion (1:40 in gel.)	−	t	t	+	+	+	++	++

Closer examination of these tables reveals a second order effect. The slight, but consistently stronger reaction of vermilion with the absorbed serum would seem to indicate that isogenic may share minor antigenic similarities with vermilion.

TABLE 4

Analysis of isogenic with respect to vermilion.

Serum: S-13, Anti-siogenic Absorbing Antigen: D-3-1, vermilion
Precipitin-Ring Tests—Hanks Technic. 24 hours readings.

ANTIGEN	TUBE							
	1	2	3	4	5	6	7	8
D-1-1, isogenic (1:12 in gel.)	++	++	++	+++	+++	+++	+++	+++
D-2-1, ruby (1:50 in gel.)	−	−	+	++	++	++	++	++
D-3-1, vermilion (1:40 in gel.)	−	−	+	++	++	++	++	++
D-1-1, isogenic (1:12 in gel.)	++	++	++	++	++	++	+++	+++
D-2-1, ruby (1:50 in gel.)	−	−	−	t	+	+	+	+
D-3-1, vermilion (1:40 in gel.)	−	−	−	t	+	+	+	+
D-1-2, isogenic (1:12 in gel.)	++	++	++	++	++	+++	+++	+++
D-2-2, ruby (1:50 in gel.)	−	−	t	t	+	+	++	++
D-3-2, vermilion (1:40 in gel.)	−	t	t	t	+	+	++	++
D-1-2, isogenic (1:12 in gel.)	++	++	++	++	++	+++	+++	+++
D-2-2, ruby (1:50 in gel.)	−	−	t	t	+	+	++	++
D-3-2, vermilion (1:40 in gel.)	−	t	t	t	+	+	++	++

The results of the double absorption of anti-isogenic serum (table 5) are, at first sight, at variance with the above results. If both mutants lack the same antigens, as is indicated above, then absorption with both simultaneously should give the same results as absorption with either singly. Yet the precipitin-ring tests, after 24 hours' incubation, gave negative results for all antigens. Only after 48 hours at 6°C are results consistent with those of the single absorptions obtained. These latter results make certain the deductions based on the single absorptions, but the delay in the reactions remains unexplained.

Antigenic analysis of vermilion.—The results obtained in the analysis of anti-vermilion serum were essentially similar to those described for anti-isogenic serum. The precipitin-ring tests indicated that upon absorption of anti-vermilion serum with increasing amounts of either isogenic or ruby antigens a point was reached where all antibodies to antigens possessed in com-

TABLE 5

Specific antigens of isogenic.

1. *Double Absorption*

Serum: S-13, Anti-isogenic
Dilution—1:2
Antigens:
D-2-1, ruby
Initial dilution—1:25
D-3-1, vermilion
Initial dilution—1:25

Incubation: 2 hrs. at 37.5°C, followed by 36 hrs. at 6°C

TUBE	1	2	3	4	5
Saline (ml)	—	0.2	0.3	0.4	0.46
D-2-1 (ml)	0.4	0.3	0.25	0.2	0.17
D-3-1 (ml)	0.4	0.3	0.25	0.2	0.17
Final dilution of each antigen	1:50	1:66.7	1:80	1:100	1:117.5
Antiserum (ml)	0.8	0.8	0.8	0.8	0.8

2. *Precipitin-Ring Tests*—Hanks Technic. 24 and 48 hours readings.

ANTIGEN	TUBE									
	24 hours					48 hours				
	1	2	3	4	5	1	2	3	4	5
D-1-1, isogenic (1:12 in gel.)	−	−	−	−	−	+	+	+	+	+
D-2-1, ruby (1:50 in gel.)	−	−	−	−	−	−	−	−	−	−
D-3-1, vermilion (1:40 in gel.)	−	−	−	−	−	−	−	−	−	t
D-1-1, isogenic (1:12 in gel.)	−	−	−	−	−	+	+	+	+	+
D-2-1, ruby (1:50 in gel.)	−	−	−	−	−	−	−	−	−	t
D-3-1, vermilion (1:40 in gel.)	−	−	−	−	−	−	−	−	−	t
D-1-2, isogenic (1:12 in gel.)	−	−	−	−	−	+	+	+	++	++
D-2-2, ruby (1:50 in gel.)	−	−	−	−	−	−	−	−	t	+
D-3-2, vermilion (1:40 in gel.)	−	−	−	−	−	−	−	−	t	+
D-1-2, isogenic (1:12 in gel.)	−	−	−	−	−	+	+	+	++	++
D-2-2, ruby (1:50 in gel.)	−	−	−	−	−	−	−	−	−	−
D-3-2, vermilion (1:40 in gel.)	−	−	−	−	−	−	−	−	−	t

mon by the three stocks were removed, leaving behind antibodies capable of precipitation with antigens specific to vermilion. There was no indication that either isogenic or ruby possessed more in common with vermilion than the other.

Double absorption of the anti-vermilion serum gave results which were in complete agreement with the results of the single absorptions. Since both heterologous antigens lacked the same fractions, the supernates, after double absorption, contained the same antibodies as they did after single absorption.

Antigenic analysis of ruby.—The results obtained with anti-ruby sera were not similar to those obtained with anti-isogenic and anti-vermilion sera. In absorbing with either of the heterologous antigens a point was reached where reaction in the precipitin-ring tests ceased simultaneously with all three antigens. These results indicated, therefore, failure to demonstrate antigenic fractions possessed by ruby which are not possessed by both of the other stocks.

Antigenic analysis of ruby vermilion.—Analysis of anti-ruby-vermilion serum yielded evidence indicating that the double mutant, ruby vermilion, shares an antigenic fraction with vermilion to the exclusion of isogenic and ruby. Identical results in the precipitin-ring tests indicated that ruby-vermilion and vermilion are antigenically identical. Despite the absence of reciprocal tests, it is reasonable to conclude that the fraction demonstrated here is identical with that already shown to be possessed by vermilion to the exclusion of ruby and isogenic.

DISCUSSION

Combination of the optimal-proportions and precipitin-ring technics has yielded results which indicate antigenic differences between the stocks employed in these experiments. For purposes of convenience, these results have been summarized, in a qualitative manner, in table 6. From these results we may draw the following conclusions. 1) Isogenic possesses an antigenic fraction specific to itself. This we shall designate as Antigen 1. 2) Vermilion possesses an antigenic fraction specific to itself. This we shall call Antigen 2. 3) No specific antigen has been demonstrated for ruby. 4) Ruby-vermilion is identical with vermilion, and therefore possesses Antigen 2. In addition to these "stock-specific" antigens, the strong cross reactions of all of the unabsorbed antisera indicate that all four stocks possess the great bulk of their antigens in common, presumably because of the identity of their residual genotypes. These antigens may be designated as "species-specific."

Can these differences be attributed to mutations at the *rb* and *v* loci, or are they associated with other genetic differences between the stocks? The answer to this question depends upon the genetic methods employed, and may be analyzed in terms of sources of genetic variability between the stocks.

Genetic variability between the stocks will depend upon the methods used in the derivation of the mutant stocks. The most important considerations here are 1) lack of control of chromosome 4, and 2) effects of the X-ray treatment.

Due to the method of their derivation, the mutant stocks would be expected

TABLE 6

Summary of results.

ANTISERUM	ABSORBED BY	TESTED WITH			
		ISOGENIC	VERMILION	RUBY	RUBY-VERMILION
Anti-isogenic	Isogenic	−	−	−	
	Vermilion	+	−	−	
	Ruby	+	−	−	
Anti-vermilion	Isogenic	−	+	−	
	Vermilion	−	−	−	
	Ruby	−	+	−	
Anti-ruby	Isogenic	−	−	−	
	Vermilion	−	−	−	
	Ruby	−	−	−	
Anti-ruby-vermilion	Isogenic	−	+	−	+
	Vermilion	−	−	−	−
	Ruby	−	+	−	+
	Ruby-vermilion	−	−	−	−

to possess, among others, a sample of fourth chromosomes derived from the isogenic stock. This being the case, isogenic antigens produced by loci on the fourth chromosome might be expected to occur in the mutant stocks as well. It will be recalled that isogenic was demonstrated to possess an antigenic fraction *not* possessed by the mutant stocks. This circumstance, along with the small size of the fourth chromosome, would seem to reduce, although not eliminate, the possibility that the antigenic differences between the stocks are due to genetic variability of the fourth chromosome.

The method of X-ray induction of visible mutations in an isogenic stock possesses the objection that invisible mutation may accompany the visible mutation and may be responsible for any observed antigenic change. The probability of such isoallelic mutation at 3,000 r is sizable, and cannot be disregarded.

Further consideration of the implications of this conclusion will be deferred until after an examination of the meaning of the antigenic changes on the hypothesis that they are due to mutation at the *rb* and *v* loci. This hypothesis is, after all, the most probable one, since mutation at these loci has definitely been demonstrated.

As a first step in the interpretation of these findings it would be well to examine the consequences of the hypothesis of a one-to-one relationship between gene and antigen in light of the genotypes of the four stocks used in this work. The genotypes of these stocks may be diagrammatically represented in the following manner:

Isogenic	*rb*$^+$	*v*$^+$	residual
Ruby	*rb*	*v*$^+$	residual
Vermilion	*rb*$^+$	*v*	residual
Ruby vermilion	*rb*	*v*	residual

If each locus produces antigens independently of the rest of the genotype, as is required by the one-to-one hypothesis, then a complete anti-isogenic serum would possess antibodies to antigens produced independently by *rb*$^+$, *v*$^+$, and each of the loci in the residual genotype. Absorption of such an antiserum by antigens of ruby would remove antibodies to the *v*$^+$ and residual antigens, leaving in the supernate antibodies to the *rb*$^+$ antigen. The supernate would, by virtue of these latter antibodies, react with isogenic and vermilion with equal strength. Similarly, absorption of the anti-isogenic serum by vermilion would leave antibodies to the *v*$^+$ antigen, and the supernate would react with isogenic and ruby with equal strength. The results of this investigation do not fit these predictions.

The best interpretation of these results is that, at least in isogenic, the *rb* and *v* loci interact to form a "compound" antigenic substance, that is, Antigen 1. Changes at either of these loci would, then, alter this substance, giving the results obtained in this investigation. It is entirely possible, of course, that other loci are involved in the production of this antigen, but since the effects of mutation of none of them have thus far been investigated, such interaction is entirely hypothetical.

A number of schemes intended to portray, in greater detail, the relationships between genes and antigens in each of the stocks are possible. Before proceeding to consideration of these schemes, implications of the possible occurrence of mutations at loci other than those of *rb* and *v* should be considered. It seems clear that regardless of this possibility the major conclusion of this paper, that is, the demonstration of genic interaction in the production of Antigen 1, remains justified. Even if the antigenic changes exhibited by the two original mutant stocks are not due to mutation at the *rb* and *v* loci, they must certainly be due to mutation at a *different* locus in each of these stocks.

Until such a time as critical work can be performed, the most economical hypothesis is that the antigenic changes observed were actually due to mutation at the *rb* and *v* loci. All further considerations will be based upon this hypothesis.

Figure 1 depicts the relationships of genes and antigens, without implication as to the mode of action of the genes or the chain of events leading to the production of the antigens. In isogenic, *rb*$^+$ and *v*$^+$ interact in an unknown fashion to produce Antigen 1, which is absent in the mutant stocks because of mutation at one or both loci. The possibility that loci of the residual genotype participate in the formation of Antigen 1 is indicated by a dotted line. Ruby possesses no specific antigens (so far as the present evidence goes)—the combination of *rb* and *v*$^+$ must, therefore, have no antigenic effect. Vermilion and the double mutant are identical in the possession of Antigen 2. This is evidently due to their common possession of *v*, which, with or without the partici-

pation of residual loci, produces Antigen 2 independently of the condition at the *rb* locus.

Figure 1 should not be considered final. The failure of the anti-ruby sera to demonstrate specific antigens in ruby should not be taken to indicate that no such fraction exists. Variation in the production of antibodies by different

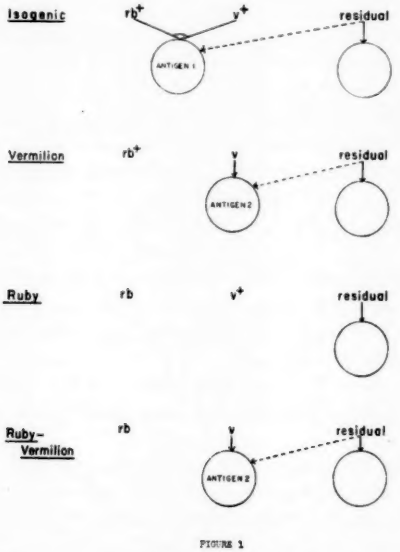


FIGURE 1

FIGURE 1.—Diagrammatic representation of relationships between genes and antigens.

rabbits is a well known phenomenon, and it may well be that the particular rabbits employed in the production of these antisera failed to form antibodies to such a specific fraction. If, upon testing additional anti-ruby sera, ruby is demonstrated to possess a specific antigenic fraction, then figure 1 will require revision.

In addition to this scheme (which implies nothing as to the physiological mechanisms of the genic control of antigen formation in this case) a number of alternative hypotheses concerned with the chain of events leading to Antigens 1 and 2, and consistent with figure 1, are possible. Figure 2 represents several such hypotheses.

The simplest hypothesis consistent with the results is that represented in figure 2a. Antigen 2 serves as substrate for the action of v^+ or its agent, being converted quantitatively to a non-antigenic substance which is in turn converted to Antigen 1 by rb^+ or its agent. Both *v* and *rb* fail to carry out the processes accomplished by their wild alleles.

If Antigen 2 be considered a product of these loci rather than a substrate for their action, then such a hypothesis as that in figure 2b becomes possible.

The same substrate is acted upon by v^+ and v, but with different results.

Figure 2c outlines a hypothesis which allows for the possibility that v^+ and v

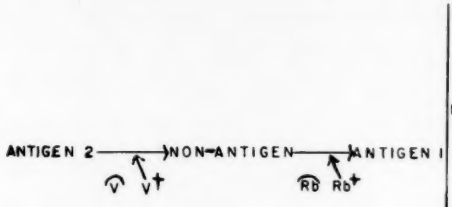


FIG. 2a

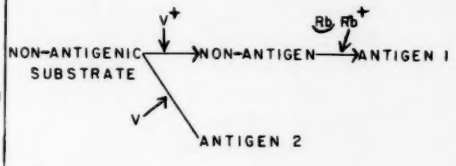


FIG. 2b

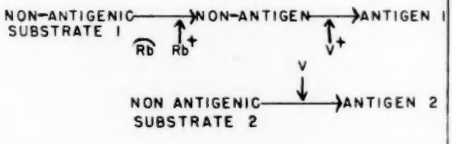


FIG. 2c

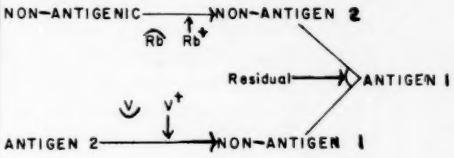
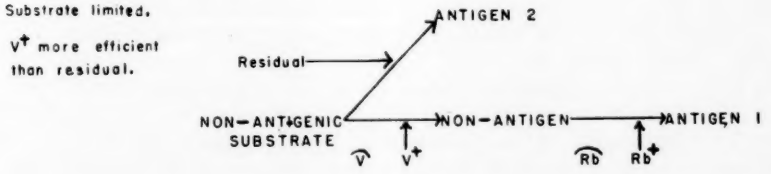


FIG. 2d



FIG, 2e

FIGURE 2.—Alternative hypotheses accounting for observed gene-antigen relationships.

act on different substrates. According to this scheme rb^+ acts on a prior link in the same chain of events as does v^+.

Should the two loci be involved in separate chains, then such a hypothesis as that in figure 2d may be formulated. Antigen 2 serves as substrate for v^+, being quantitatively converted to Non-antigen 1. A non-antigenic substrate

is converted to Non-antigen 2 by rb^+. Non-antigens 1 and 2 are combined by one or more loci in the residual genotype, or spontaneously, to form Antigen 1.

These four hypotheses all assume that the availability of substrate is not a limiting factor, and that the rate-constants of the various gene-controlled reactions (WRIGHT 1941) or the efficiency of the various genes (STERN 1943) do not differ. Other hypotheses may consider the possibility of limited substrate and different rate-constants or efficiencies. In figure 2e a non-antigenic substrate, possessing limited availability, may be utilized by both v^+ and one or more loci in the residual genotype. The rate-constant of the reaction controlled by v^+ is, however, greater than that of the reaction controlled by the residual genotype (that is, the efficiency of v^+ is greater than that of residual). Under these circumstances, Antigen 2 will not be formed in the presence of v^+; in the absence of v^+ Antigen 2 will be formed.

It is impossible, on the basis of the data at hand, to judge objectively the relative validity of these or equally likely hypotheses. It is nevertheless interesting to note the complexity of gene-product relationships encountered in a field which previously had been considered one of almost unique simplicity.

The relation between the results of this investigation and the known effects of the *rb* and *v* loci on eye-color is obscure. CASPARI (1946) has demonstrated that the gene *a* in *Ephestia kühniella*, the homologue of *v* in Drosophila, has the effect of causing the accumulation of tryptophane in the tissues, and the incorporation of that amino acid in proteins to a greater degree than in a^+/a^+. This difference in protein structure should have a reflection in antigenic specificity. Unfortunately, in Drosophila *v* does not cause a corresponding change in protein tryptophane, although it does increase the amount of free tryptophane (GREEN 1948 and personal communication).

The conversion of tryptophane to kynurenin under the influence of v^+ is well known (EPHRUSSI 1942). SEWALL WRIGHT (personal communication) has suggested that if kynurenin is a precursor of Antigen 1, then the production of Antigen 2 in the absence of v^+ but not in the absence of rb^+ means that v^+ and rb^+ act on different steps. His suggestion is in agreement with several of the schemes presented above.

The frequency of occurrence of genic interaction in the production of antigens can be determined only by investigation of a large number of other loci. Nevertheless, the possibility that this is a wide-spread phenomenon should be considered. It would be expected that any mutation affecting a biochemical synthesis, more particularly the synthesis of an amino acid, should have an effect on protein structure. Loci involved in a chain of reactions leading to the production of an amino acid would, then, be involved in genic interaction in the production of antigens. This is the case in bacteria, where biochemical and phage-resistance mutations are frequently accompanied by antigenic changes (LURIA 1947). It would be surprising if the same situation did not prevail in higher organisms.

In conclusion, a possible explanation for the difference in the evolutionary behavior of haptenic and protein antigens might be proposed. If it should be found that genic interaction is the rule in the production of proteins in contrast

to a one-to-one relation between gene and hapten, then the sudden, intraspecific changes in haptens would be attributable to mutations in the single genes involved in their production while the gradual variation of proteins would be easily understood on the basis of the many genes involved.

SUMMARY

1. An isogenic strain of *D. melanogaster*, two derived strains differing from the isogenic by X-ray induced mutations, one at the *v* locus and one at the *rb* locus, and a double mutant derived from the two latter strains have been subjected to antigenic analysis.

2. Anti-isogenic serum, when absorbed by vermilion antigens, still reacts strongly with isogenic antigens, but with neither vermilion nor ruby antigens. The same serum, when absorbed by antigens of the ruby strain, still reacts strongly with the isogenic antigens, reacts very weakly with vermilion antigens, and not at all with ruby antigens. These results indicate an antigenic component possessed by isogenic but not by either mutant.

3. Somewhat similarly, anti-vermilion serum, when absorbed by either isogenic or ruby antigens, still reacts strongly with vermilion antigens, but with neither isogenic nor ruby antigens. Vermilion, therefore, possesses an antigenic component specific to itself in relation to ruby and isogenic.

4. To date, anti-ruby sera have failed to demonstrate antigenic components specific to ruby, not possessed by isogenic and ruby.

5. Anti-ruby-vermilion serum gives results identical with those of anti-vermilion serum. The double mutant is concluded to be antigenically similar to vermilion.

6. Double absorptions of the various anti-sera give results consistent with those obtained by single absorptions.

7. These results may be taken to indicate a more complex situation than would be expected on the hypothesis of a one-to-one relationship between gene and antigen. At least the *rb* and *v* loci seem to be involved in the production of an antigen or antigenic complex by means of interaction. Several alternative hypotheses, consistent with the data, are discussed.

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CHROMATID SEGREGATION OF TETRAPLOIDS AND HEXAPLOIDS[1]

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INTRODUCTION

IN an earlier paper (GEIRINGER 1948) the author has investigated the mathematical genetics of autopolyploids if one locus is considered and chromosome segregation is assumed. A paper on the linkage theory of autopolyploids (m loci), under the same assumption, is in press (GEIRINGER 1949). However, while chromosome segregation might be assumed as an approximation theory, the study of polyploids should actually be based on the consideration of chromatid segregation. In the case of one locus this theory can be worked out and is presented in this paper as far as random mating is concerned. This study, for $m=1$, as well as the approximation theory for general m, may serve as a necessary preparation for a general linkage theory of polyploids under chromatid segregation.

A basic paper by J. B. S. HALDANE (1930) is mainly concerned with the chromosome segregation theory of polyploids; "random chromatid segregation" of tetraploids is briefly discussed too and a limit formula (our formula (24)) follows. In a paper of a more recent date R. A. FISHER (1947) deals with the linkage theory of polyploids under chromatid segregation. The paper uses important earlier investigations by the same author (1941, 1944) as well as a paper by FISHER and MATHER (1943) and papers by MATHER (1935, 1936).

As in previous papers it is our aim to consider a heredity problem like the one in question as a probability problem. From certain basic probability distributions which must be known other genetical distributions are derived by means of probability calculus. The fate of these distributions, from generation to generation, is the main subject of the mathematical investigation.

If we assume distinct, non overlapping generations, numbered 0, 1, $\cdots$ n, $\cdots$ we may denote by $w^{(n)}(x; y)$ the *distribution of genotypes* in the n^{th} generation. Here x denotes the genetic material the individual has received from its mother and y designates the paternal heritage. We assume that there is no genotypic difference between an individual of type (x; y) and one of type (y; x); that is: $(x; y)=(y; x)$. Accordingly $w^{(n)}(x; y)=w^{(n)}(y; x)$; and $\sum_x\sum_y w^{(n)}(x; y)=1$. We may assume that at the beginning, for $n=0$, the distributions of genotypes are the same for males and females; if they are not the same, that is, if $w^{(0)}(x; y)$ is the genotype for females and $\bar{w}^{(0)}(x; y)$ the one for males, they will be the same after one generation of random breeding.

In the formation of a new individual a parent transmits to the offspring one set of genes, the other set coming from the other parent. The kinds of gametes which an organism may produce correspond to the possible combinations of the genetic material it has inherited. This segregation takes place according to a

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probability law which we call *segregation distribution* (s.d., see section 2). It is one of the main tasks of biological theory to suggest, in accordance with observations and other theoretical (for instance cytological) evidence, the s.d. which corresponds to a biological situation. If we assume random breeding, it is possible to derive from the *distribution of genotypes* (d.ge.) and the s.d. the third important distribution, the *distribution of gametes* (d.ga.), $p^{(n)}(z)$. Finally, under random mating, $w^{(n+1)}(x; y)$ follows from $p^{(n)}(z)$ since a new genotype is formed by the fusion of two gametes. A complete cycle of inheritance is thus described.

So far we have been faced with the following problems: a) Complete enumeration of the possible genotypes and gametes corresponding to a biological situation; b) definition of the s.d.; c) derivation of the d.ga. from the d.ge. by means of the s.d. These problems are of course not new: for example, FISHER's statement "The laws of inheritance obtained by genetic studies are the rules whereby, given the constitution of an organism, the kinds of gametes it can produce and their relative frequencies can be predicted" (1947, p. 55) corresponds to our problems b) and c). While in all these instances the basic suggestions must come from the biologist—from his observations and their interpretation—the mathematician may be able to help in clarifying and simplifying the concepts.

A few further problems are of a more theoretical nature: d) It is essential to derive direct *recurrence relations* between subsequent distributions of gametes, that is, relations by means of which we know $p^{(n+1)}(z)$ if $p^{(n)}(z)$, or perhaps several $p^{(\gamma)}(z)$ where $\gamma \leqq n$, are known. Such a recurrence formula will be simpler than the corresponding one for $w^{(n+1)}(x; y)$. The recurrence problem constitutes the basic problem of the theory. e) It is desired to *integrate* these recurrence relations, that is, to determine $p^{(n)}(z)$ in terms of $p^{(0)}(z)$, of "n," and of the parameters introduced. f) We want to know whether, and if so, under what conditions an *equilibrium status* is reached for $p^{(n)}(z)$, and, consequently, for $w^{(n)}(x; y)$. In connection with this question we must study the *limit distribution* of $p^{(n)}(z)$ and of $w^{(n)}(x; y)$ as $n \to \infty$.

Those last problems seem to be of a rather theoretical character. They are, however, of practical value as well, since they allow qualitative and quantitative predictions which, in turn, may be checked by means of observations. In particular, the equilibrium distribution is of interest, since we may often be entitled to assume that a population has actually reached the equilibrium status.

In the present paper all these problems have been studied for autopolyploids with *one locus*, for an *arbitrary number of alleles*, r, assuming *chromatid segregation*. The result is that we find an entirely *new type of recurrence relations* as compared with those in GEIRINGER (1944, 1948) which are characteristic for chromosome segregation; we likewise arrive at a *new type of limit distribution* (see sections 3 and 5). The case of tetraploids and hexaploids has been investigated completely and octoploids have been considered in some detail. Let us now, briefly, describe the approach.

The main characteristic of a polyploid organism is that (with respect to one locus) a gamete consists not of one but of $s > 1$ genes. An orthopolyploid has $2s$ genes, or $2s$ chromosomes. More specifically, the organism possesses two sets

of s chromosomes each, each chromosome being represented by one of the numbers $a_1, \cdots, a_r$, the r alleles, where r may be less than, greater than, or equal to s. Accordingly, the d.ge. and the d.ga. are discrete probability distributions in 2s and s variables respectively. In the formation of a new individual each parent transmits to the offspring one set of s genes. The selection of those transmitted genes happens according to the s.d. Of this s.d. we assume: 1) that it is the same for males and females, 2) that it does not depend on "n" and hence remains the same throughout the generations, 3) that it does not depend on the specific genotype of the parent.

There are 4s chromatids (2s chromosomes) and out of these 4s chromatids,

$$S = \binom{4s}{s} = \frac{(4s)!}{s!(3s)!}$$

sets of s can be selected. For $s = 2, 3, 4$, $S = 28, 220, 1820$. (See page 671 for comparison with chromosome segregation theory.) A set of s selected genes may be derived from $(s-\rho)$ different chromosomes where ρ may take on the values $0, 1, \cdots, \mu$ if $s = 2\mu$ or $2\mu+1$ respectively; such a set contains ρ pairs of sister chromatids. Hence there are from this point of view $(\mu+1)$ different modes of segregation. A gamete may be called "normal" for the moment if it is derived from s chromosomes ($\rho = 0$) and hence does not contain any pair of sister chromatids. There exist obviously $S_1 = 2^s \cdot \binom{2s}{s}$ "normal" gametes and $S - S_1$ gametes which contain at least one pair of sister chromatids (due to "double reduction"). If the consequences of occasional double reduction are neglected, that is, if in the definition of the s.d. all probabilities of non-normal gametes are assumed to be zero, then chromatid s.d. and chromosome s.d. amount to the same. Such an assumption may be regarded as an approximation to reality. According to the $(\mu+1)$ values of ρ, FISHER and MATHER introduce $(\mu+1)$ parameters in the s.d. (section 2).

The present author considers a more general s.d. Besides the above explained differentiation, which refers to the values of ρ, we take into account the *proportion of paternal and of maternal genes* in a gamete. This idea has been used to great advantage in the author's previous papers (1944, 1948, 1949). In fact, this more differentiated segregation distribution is needed in order to establish the recurrence relations and to understand their structure. In these recurrence formulas which form the main aim of the mathematical theory and which express biological facts, the values of the s.d. act as separators between meaningful groups of probabilities. This fact suggests that these parameters may have a biological meaning; this, of course, can be checked by counting the results of observations and evaluating the resulting figures in the usual statistical way. On the other hand, it is very easy to forget about those parameters after having established the recurrence relations. If, for example, we accept FISHER and MATHER's segregation theory we merely have to put all our segregation probabilities which correspond to the same ρ equal to each other, reducing thus the number of parameters to $(\mu+1)$. Other s.d.'s contained as particular cases in our s.d. represent *the general chromosome s.d.* of the author's earlier paper (1948), *random chromatid segregation* (HALDANE 1930), etc.

In section 2 of this paper the three basic distributions, the distributions of genotypes and of gametes, and the segregation distribution are defined. In section 3 we derive the recurrence relations (problem (d)) for $s=2, 3, 4$. They differ in an essential way from the recurrence relations under chromosome segregation. Section 4 contains, by way of illustration, a simple numerical example. Finally, in section 5 corresponding to problems e) and f), the recurrence relations are integrated and the equilibrium status is determined.

THE DISTRIBUTION OF GAMETES, THE DISTRIBUTION OF GENOTYPES, AND THE SEGREGATION DISTRIBUTION

The enumeration of the possible types of gametes and of genotypes is simple. In the case of $m=1$ locus there exist, for a 2s-ploid and r alleles, $R=\binom{r+s-1}{s}$ possible gametes; that is, if $s=2$, $r=3$: $R=6$ and the gametes are a_1^2, a_2^2, a_3^2, a_1a_2, a_2a_3, a_3a_1; or if $s=4$, $r=5$: $R=70$ possible gametes: $a_1^4, \cdots a_5^4$, $a_1^3a_2, \cdots, a_4^3 a_5, \cdots, a_2a_3a_4a_5$. If one wants to enumerate the possible *types* of gametes, the number of *partitions* is to be considered as R. A. FISHER remarks. Denote by $\pi_\rho(s)$, $(\rho \geqq 1)$ the number of different ways in which the integer s can be resolved into the sum of ρ positive integers; clearly $\pi_\rho(s)=0$, if $\rho > s$. Next put $P_r(s)=\sum_{\rho=1}^{r}\pi_\rho(s)$. There are obviously $P_r(s)$ different types of gametes for a 2s-ploid and r alleles. Consider for instance $s=2$, $r=3$ $\pi_1(2)=1$, $(2=2)$, $\pi_2(2)=1$, $(2=1+1)$, $\pi_\rho(2)=0$, $\rho \geqq 3$; $P_3(2)=1+1+0=2$ and the two types are, in fact: a_1^2 and a_1a_2. Or if $s=4$, $r=3$: $\pi_1(4)=1$, $(4=4)$, $\pi_2(4)=2$, $(4=3+1=2+2)$, $\pi_3(4)=1$, $(4=2+1+1)$, $\pi_4(4)=1$, $(4=1+1+1+1)$, $\pi_\rho(4)=0$, $\rho \geqq 5$; and $P_3(4)=1+2+1=4$. In fact the types are: a_1^4, $a_1^3a_2$, $a_1^2\,a_2^2$, $a_1^2a_2a_3$.

Similar remarks hold for the genotypes. If (like most authors) one does not distinguish between maternal and paternal heritage there are $R'=\binom{r+2s-1}{2s}$ possible genotypes. If, however, this distinction *is* made, (as we do), then, with $R=\binom{r+s-1}{s}$ there are $R(R+1)/2$ possible genotypes.

For example for $2s=4$, $r=4$ these two numbers are 35 and 55 respectively with 5 or 7 different types respectively. These are in the first conception: a_1^4, $a_1^3a_2$, $a_1^2a_2^2$, $a_1^2a_2a_3$, $a_1a_2a_3a_4$, and in the second: $(a_1^2; a_1^2)$, $(a_1^2; a_1a_2)$, $(a_1^2; a_2^2)$, $(a_1a_2; a_1a_2)$, $(a_1^2; a_2a_3)$, $(a_1a_2; a_1a_3)$, $(a_1a_2; a_3a_4)$.

Let us review a few of the definitions given in the author's previous paper (1948), particularly those dealing with the *distribution of gametes* and the *marginal distributions* derived from it. For $s=2$ the d.ga. is given by $p(z_1, z_2)$, (we may omit the upper script) where each of the two z_i may take on each of the r values $a_1, \cdots, a_r$. We assume that there is no difference between the gametes (a_ia_k) and (a_ka_i) and consequently, $p(a_ia_k)=p(a_ka_i)$. Hence there is one value $p(a_i^2)$ but two values $p(a_ia_k)$, $(a_i \neq a_k)$ and

$$\sum_{i}^{1\cdots r} \sum_{k}^{1\cdots r} p(a_i a_k) = 1.$$

From $p(z_1 z_2)$ we derive the marginal distributions of order one:

$$p_1(a_j) = \sum_{z}^{a_1 \cdots a_r} p(a_j, z) \quad \text{and} \quad p_2(a_j) = \sum_{z}^{a_1 \cdots a_r} p(z, a_j), \quad (j = 1, 2, \cdots, r).$$

It follows from $p(a_i a_k) = p(a_k a_i)$ that $p_1(a_j) = p_2(a_j) = p(a_j)$; these are the ***marginal distributions of order one*** and

$$\sum_{i}^{1\cdots r} p(a_i) = 1.$$

If $s=3$ the d.ga. is $p(z_1 z_2 z_3)$ where each z_i equals a_1, or a_2, $\cdots$, or a_r and we derive from it

$$p_{12}(a_i a_k) = \sum_{z}^{a_1 \cdots a_r} p(a_i, a_k, z), \qquad p_{13}(a_i a_k) = \sum_{z}^{a_1 \cdots a_r} p(a_i, z, a_k)$$

and $p_{23}(a_i a_k)$. Again $p_{12}(a_i a_k) = p_{13}(a_i a_k) = p_{23}(a_i a_k) = p(a_i a_k)$. This ***marginal distribution of order two*** denotes the probability that of the existing three genes two (specific) genes have the values a_i and a_k. In the same way $p_1(a_i) = p_2(a_i) = p_3(a_i) = p(a_i)$ is derived from $p(z_1, z_2, z_3)$ and

$$\sum \sum \sum p(a_i, a_j, a_k) = 1, \qquad \sum \sum p(a_i a_j) = 1, \qquad \sum p(a_i) = 1.$$

For any s (see GEIRINGER 1948, 271ff) the d.ga. may be denoted either by $p(z_1 \cdots z_s)$, $(z_i = a_1 \cdots, a_r, i = 1, 2, \cdots, s)$, or by $p(a_1^{x_1} a_2^{x_2} \cdots a_r^{x_r})$, $(x_j = 0, 1, 2, \cdots; x_1 + x_2 + \cdots + x_r = s)$ and marginal distributions of orders, $1, 2, \cdots, s-1$ may be derived; for instance with

$$s = 7, r = 5, p(a_1^2 a_2) = \sum_{z_1} \sum_{z_2} \sum_{z_3} \sum_{z_4} p(a_1 a_1 a_2 z_1 z_2 z_3 z_4)$$

(the summations are from a_1 to a_5), and there are $\binom{7}{3} \cdot \binom{3}{2} = 105$ such probabilities; here $p(a_1^2 a_2)$ denotes the probability that of the existing 7 genes, 3 specified ones have the values a_1, a_1, a_2.

All this is independent of the distinction between chromosome and chromatid segregation.

Let us now consider *the s.d.* In a 2s-ploid of one locus, there are 2s chromosomes and 4s chromatids; in a diploid organism there are 2 chromosomes and 4 chromatids. *For the diploid with any number of loci, chromatid segregation and chromosome segregation amounts to the same.* In fact denote a diploid with m loci by $(x; y) = (x_1 \cdots x_m; y_1 \cdots y_m)$ where x_i, (y_i) constitutes the maternal (paternal) heritage with respect to the i^{th} character. Consider chromosome segregation. There are 2^m possible gametes. We denote (GEIRINGER 1944) for instance by $1(11000 \cdots 10)$ the probability that $(x_1 x_2 y_3 y_4 y_5 \cdots x_{m-1}, y_m)$ be transmitted—with certain symmetry relations for these $1(\epsilon_1 \epsilon_2 \cdots \epsilon_m)$. Next

consider chromatid segregation and denote by x_i, x_i' or by y_i, y_i' corresponding sister chromatids. There are now $2^m \cdot 2^m = 2^{2m}$ possible gametes. Denote by $2^m l'(11 \cdots 1)$ the probability that the gamete contains x-values only (for example, $(x_1 x_2 \cdots x_m)$, or $(x_1' x_2 \cdots x_m)$, etc.) by $2^m l'(11000 \cdots 10)$ the probability that the first, second $\cdots$ and $(m-1)^{st}$ value is an x, the third, fourth, fifth $\cdots$ and last a y-value, etc. Then, with $2^m l'(\epsilon_1 \epsilon_2 \cdots \epsilon_m) = l(\epsilon_1 \epsilon_2 \cdots \epsilon_m)$, $(\epsilon_i = 0, 1; i = 1, 2, \cdots, m)$ we obtain again the same s.d. as before. The reason is of course that, if $s = 1$, with respect to each character, one gene only is transmitted, hence x_i, x_i' can not be found in the same gamete. In particular, *there is no difference between* "random chromosome segregation" ("independent assortment") and "random chromatid segregation." The former amounts to

$$l(\epsilon_1 \epsilon_2 \cdots \epsilon_m) = \frac{1}{2^m}$$

for all ϵ-combinations, the second to

$$l'(\epsilon_1 \epsilon_2 \cdots \epsilon_m) = \frac{1}{2^{2m}}$$

and

$$2^m l'(\epsilon_1 \cdots \epsilon_m) = \frac{1}{2^{2m}} \cdot 2^m = \frac{1}{2^m} = l(\epsilon_1 \epsilon_2 \cdots \epsilon_m).$$

This is quite different if $s > 1$. Assume $m = 1$ (one locus), and denote an organism by $(x_1 \cdots x_s; y_1 \cdots y_s)$ where each x_i and each y_i may be equal to one of the r alleles $a_1, \cdots, a_r$. If we want a notation to show the 4s chromatids we write:

$$(1) \qquad (x_1 \cdots x_s, x_1' \cdots x_s'; y_1 \cdots y_s, y_1' \cdots y_s')$$

or rather:

$$(1') \qquad \begin{pmatrix} x_1 \cdots x_s & y_1 \cdots y_s \\ x_1 \cdots x_s & y_1 \cdots y_s \end{pmatrix}.$$

Denote by μ_α the probability that a specified set of α maternal and a specified set of $(s-\alpha)$ paternal genes be transmitted, but such that the resulting gamete does not contain a single pair of sister chromatids. There are, corresponding to each α, $2^\alpha \binom{s}{\alpha} \cdot 2^{s-\alpha} \binom{s}{s-\alpha} = 2^\alpha \left[\binom{s}{\alpha}\right]^2$ such probabilities and we assume *that they be all equal to each other;* moreover we assume

$$(2) \qquad \mu_\alpha = \mu_{s-\alpha}.$$

Next *denote by* μ_α' (by μ_α'', by μ_α''', $\cdots$,) *the probability that a specified set of α maternal and $(s-\alpha)$ paternal genes be transmitted such that the resulting gamete contains exactly one pair (exactly two pairs, exactly three pairs, $\cdots$) of corresponding sister chromatids.* In other words: The gamete is derived from $s-\rho$ different chromosomes where $\rho = 1, 2, \cdots, \mu$, if $s = 2\mu$ or $2\mu+1$ respec-

tively. Assume that all μ_α' for the same α, equal each other; all μ_α'' for the same α, equal each other; etc. and

$$\mu_\alpha' = \mu'_{s-\alpha},\ \mu_\alpha'' = \mu''_{s-\alpha},\ \text{etc.} \tag{3}$$

With these notations we have for s=2, 3, 4:

$$s = 2: \quad 4(\mu_0 + 4\mu_1 + \mu_2) + 2(\mu_0' + \mu_2') = 1 \tag{4}$$

or, using (2), (3):

$$s = 2: \quad 4\cdot 2(\mu_0 + 2\mu_1) + 4\mu_0' = 1 \tag{4'}$$

$$s = 3: \quad 8\cdot 2(\mu_0 + 9\mu_1) + 2(12\mu'_0 + 18\mu_1') = 1 \tag{5}$$

$$s = 4: \quad 16\cdot 2(\mu_0 + 16\mu_1 + 18\mu_2) + 2(48\mu_0' + 192\mu_1' + 96\mu_2') + 2(6\mu_0'' + 8\mu_2'') = 1. \tag{6}$$

We see that for s=2, 3, 4 we have respectively 2, 3, and 7 parameters. According to FISHER and MATHER (1943) we would put for each s, all μ equal to each other, all μ' equal to each other, etc. and this would amount to:

$$s = 2: \quad 24\mu + 4\mu' = 1, \quad 4\mu' = \alpha \tag{4''}$$

$$s = 3: \quad 160\mu + 60\mu' = 1, \quad 60\mu' = \beta \tag{5''}$$

$$s = 4: \quad 1120\mu + 672\mu' + 28\mu'' = 1, \quad 28\mu' = \gamma, \quad 28\mu'' = \delta. \tag{6''}$$

If the s.d. is known, the "gametic output" that corresponds to any particular genotype follows very easily. As an example consider the genotype

$$(a_1a_2^2;\ a_1^2a_3) \quad \text{or} \quad \begin{pmatrix} a_1a_2a_2 & a_1a_1a_3 \\ a_1a_2a_2 & a_1a_1a_3 \end{pmatrix}$$

where the following ten gametes and their corresponding probabilities may originate:

gamete	probability
a_1^3	$4\mu_2' + 8\mu_1 + 4\mu_1' + 4\mu_0'$
a_2^3	$4\mu_3'$
a_3^3	0
$a_1^2a_2$	$4\mu_3' + 32\mu_2 + 16\mu_1 + 8\mu_1$
$a_1^2a_3$	$2\mu_2' + 16\mu_1 + 8\mu_0 + 4\mu_0'$
$a_2^2a_1$	$4\mu_3 + 4\mu_3' + 16\mu_2 + 8\mu_2'$
$a_2^2a_3$	$8\mu_2 + 4\mu_2'$
$a_3^2a_1$	$2\mu_1' + 4\mu_0'$
$a_3^2a_2$	$4\mu_1'$
$a_1a_2a_3$	$16\mu_2 + 32\mu_1$

To illustrate the computation of each of these ten probabilities consider for instance $a_1^2a_2$: Firstly, three maternal values may be transmitted, namely the two a_1-values and one of the four a_2-values; that can happen in $1 \cdot 4 = 4$ ways and since in such a gamete one pair of sister chromatids occurs the probability is $4\mu_3'$. Next, two maternal values and one paternal value may be used; there are to the left of the semicolon two a_1-chromatids and four a_2-chromatids, hence a_1a_2 may be selected in eight ways while the last a_1-value may be selected from the four values to the right in four ways, hence the term $32\mu_2$. Next one maternal value is used; since a_2 occurs to the left only this value must be a_2, which can be selected in four ways; it is combined with a pair out of the four a_1-values to the right. Among the six possible pairs of such values two correspond to sister-chromatids while four pairs are "normal"; hence the two terms $4 \times 4\mu_1 + 4 \times 2\mu_1'$. In the same way all other gametes and their probabilities may be discussed.

If the genotype in question is considered as: $(a_1^3a_2^2a_3)$ and accordingly all μ-values are equal to each other and all μ'-values equal to each other, the probability of the gamete $a_1^2a_2$ becomes

$$48\mu + 12\mu' + \frac{18 - 6\beta}{60}$$

which appears in FISHER, 1947, p. 58, second table, second line, third column. (Our a_1, a_2, a_3 are denoted by Fisher by a, A′, A).

Consider on the other hand the genotype $(a_1a_2a_3;\ a_1^2a_2)$ which, in my conception is different from $(a_1a_2^2;\ a_1^2a_3)$. The probability that the organism $(a_1a_2a_3;\ a_1^2\,a_2)$ transmits the gamete $a_1^2a_2$ is: $2\mu_3' + 16\mu_2 + 2\mu_2' + 16\mu_3 + 8\mu_1 + 4\mu_1' + 8\mu_0 + 4\mu_0'$ and this is quite different from the previously found value $4\mu_3' + 32\mu_2 + 16\mu_1 + 8\mu_1'$ which corresponds to the same gamete $(a_1^2a_2)$ derived from $(a_1a_2^2;\ a_1^2a_3)$. If however, $\mu_3 = \mu_2 = \mu_1 = \mu_0$, $\mu_3' = \mu_2' = \mu_1' = \mu_0'$ the preceding probability reduces again to $48\mu + 12\mu'$, as before. These examples should suffice. (See also beginning of sec. 4.)

As stated before, *our* s.d. *reduces to* FISHER *and* MATHER'S *assumption by equating all* μ *to each other, all* μ' *to* each other, all μ'' to each other, etc. It *reduces to the general chromosome segregation distribution* introduced in GEIRINGER (1948) if all μ', all μ'', $\cdots$ *are equal to zero.* In particular, *random chromosome* segregation follows if, *in addition all* μ_α *are equal to* each other. We have *random chromatid segregation* if *all* parameters, that is all μ_i, μ_j', μ_k'', $\cdots$, are equal to each other.

RECURRENCE FORMULAS IN CASE OF S = 2, 3, 4

An important formal justification for the introduction of our more complicated s.d. may be found in the role which this s.d. plays in the recurrence formulas which, in the author's opinion, constitute the main result of the mathematical theory. These recurrence formulas, based on the consideration of chromatid segregation, are essentially different from the ones that hold for polyploids with one or several loci if chromosome segregation is assumed. The

new recurrence formulas are *no longer homogeneous*, in a sense we shall explain presently. Nevertheless they are still quite clear-cut and understandable.

We know that if all μ_j', μ_k'', $\cdots$ are equal to zero, chromatid segregation reduces to chromosome segregation. Hence in this case the new recurrence formulas must reduce to the known recurrence formulas for chromosome segregation (GEIRINGER 1948). Hence we actually knowthese new formulas as far as the coefficients of the μ_i are concerned and we are interested in the other terms only.

Let us start with $s=2$. Use $v(x;y)=w(x;y)+w(y;x)=2w(x;y)$, $(x\neq y)$, $v(x;x)=w(x;x)$. In terms of these v we have $v(a_1^2;a_1^2)+2v(a_1^2;a_1a_2)+v(a_1^2;a_2^2)+4v(a_1a_2;a_1a_2)+2v(a_1a_2;a_2^2)+v(a_2^2;a_2^2)+\cdots=1$. Here the points at the end of the left side indicate terms corresponding to other alleles. We write $p^{(n)}=p$, $v^{(n)}=v$, $p^{(n+1)}=p'$, $v^{(n+1)}=v'$. We assume now two alleles and compute in the same way as in the example page 671, the probability of the gamete (a_1^2) as derived from various genotypes:

$$\begin{aligned} p(a_1^2) = {} & v(a_1^2;a_1^2)\cdot 1 + 2v(a_1^2;a_1a_2)(\mu_2' + 8\mu_1 + 4\mu_0 + 2\mu_0') \\ & + v(a_1^2;a_2^2)(4\mu_0 + 2\mu_0') + 4v(a_1a_2;a_1a_2)(\mu_2' + 4\mu_1 + \mu_0') \\ & + 2v(a_1a_2;a_2^2)\mu_0'. \end{aligned} \tag{7}$$

Next we write the same formula (7) for the $(n+1)^{st}$ generation and consider:

$$w^{(n+1)}(x;y) = p^{(n)}(x)p^{(n)}(y) \tag{8}$$

we get, using (2) and (3):

$$\begin{aligned} p'(a_1^2) = {} & p(a_1^2)^2 + p(a_1^2)p(a_1a_2)(12\mu_0' + 16\mu_0 + 32\mu_1) \\ & + p(a_1^2)p(a_2^2)(8\mu_0 + 4\mu_0') + p(a_1a_2)^2(16\mu_1 + 8\mu_0') \\ & + p(a_1a_2)p(a_2^2)\cdot 4\mu_0' \\ = {} & 8\mu_0\cdot[p(a_1^2)^2 + 2p(a_1^2)p(a_1a_2) + p(a_1^2)p(a_2^2)] \\ & + 16\mu_1[p(a_1^2)^2 + 2p(a_1^2)p(a_1a_2) + p(a_1a_2)^2] \\ & + 4\mu_0'[p(a_1^2)^2 + 3p(a_1^2)p(a_1a_2) + p(a_1^2)p(a_2^2) \\ & + 2p(a_1a_2)^2 + p(a_1a_2)p(a_2^2)]. \end{aligned}$$

The expressions in brackets are respectively:

$$\begin{aligned} & p(a_1^2)\cdot[p(a_1^2) + 2p(a_1a_2) + p(a_2^2)] = p(a_1^2)\cdot 1 = p(a_1^2) \\ & [p(a_1^2) + p(a_1a_2)]^2 = [p(a_1)]^2 \\ & [p(a_1^2) + 2p(a_1a_2) + p(a_2^2)]\cdot[p(a_1^2) + p(a_1a_2)] = 1\cdot p(a_1) = p(a_1). \end{aligned}$$

Thus we get:

$$p^{(n+1)}(a_1^2) = 8\mu_0 p^{(n)}(a_1^2) + 16\mu_1[p^{(n)}(a_1)]^2 + 4\mu_0' p^{(n)}(a_1). \tag{9}$$

We find the same formula (9) without any change if we assume, more generally, $r>2$ alleles.

This formula (9) reduces to my formula for chromosome segregation if

$\mu_0'=0$, $4\mu_0=\lambda_0$, $4\mu_1=\lambda_1$. If, however, $\mu_0'\neq 0$, (9) is a *non-homogeneous* recurrence formula in contrast to all recurrence formulas established (in GEIRINGER 1944, 1948) under the assumption of chromosome segregation. In fact $p^{(n+1)}(a_1^2)$, $p^{(n)}(a_1^2)$, and $[p^{(n)}(a_1)]^2$ are "of second degree" while $\mu_0' p^{(n)}(a_1)$ is "of first degree." The occurrence of such a term in the recurrence formula can easily be interpreted. This will be done later, together with the consideration of the cases $s=3$, and $s=4$.

Next we compute $p^{(n+1)}(a_1a_2)$ and find:

$$p^{(n+1)}(a_1a_2) = 8\mu_0 p^{(n)}(a_1a_2) + 16\mu_1 p^{(n)}(a_1)p^{(n)}(a_2). \tag{9'}$$

Upon addition of (9) and (9') we obtain:

$$p^{(n+1)}(a_1) = 8\mu_0 p^{(n)}(a_1) + 16\mu_1 p^{(n)}(a_1) + 4\mu_0' p^{(n)}(a_1) = p^{(n)}(a_1).$$

Hence, just as in case of chromosome segregation:

$$p^{(n+1)}(a_i) = p^{(n)}(a_i) = p^{(0)}(a_i) \qquad \begin{matrix}(n = 1, 2, \cdots)\\ (i = 1, 2, \cdots r)\end{matrix} \tag{10}$$

The frequency of each allele remains constant throughout the generations. None of these results changes in case of any number r of alleles. Thus the recurrence formulas for $s=2$ become (with $p^{(0)}(a_i)\equiv p(a_i)$):

$$\begin{aligned} p^{(n+1)}(a_i^2) &= 8\mu_0 p^{(n)}(a_i^2) + 16\mu_1[p(a_i)]^2 + 4\mu_0' p(a_i) \\ p^{(n+1)}(a_ia_k) &= 8\mu_0 p^{(n)}(a_ia_k) + 16\mu_1 p(a_i)p(a_k) \qquad (i, k = 1, 2, \cdots, r). \end{aligned} \tag{9''}$$

For *random chromatid segregation* $\mu_1=\mu_0=\mu_0'=\frac{1}{28}$ these become:

$$\begin{aligned} p^{(n+1)}(a_i^2) &= \frac{2}{7} p^{(n)}(a_i^2) + \frac{4}{7}[p(a_i)]^2 + \frac{1}{7} p(a_i) \\ p^{(n+1)}(a_ia_k) &= \frac{2}{7} p^{(n)}(a_ia_k) + \frac{4}{7} p(a_i)p(a_k) \end{aligned} \tag{9'''}$$

To "complete *equational separation*" (MATHER 1936) $\mu_0=\mu_1=\frac{5}{144}$, $\mu_0'=\frac{6}{144}$ correspond the formulas:

$$\begin{aligned} p^{(n+1)}(a_i^2) &= \frac{5}{18} p^{(n)}(a_i^2) + \frac{10}{18}[p(a_i)]^2 + \frac{3}{18} p(a_i) \\ p^{(n+1)}(a_ia_k) &= \frac{5}{18} p^{(n)}(a_ia_k) + \frac{10}{18} p(a_i)p(a_k). \end{aligned} \tag{9IV}$$

Under FISHER-MATHER'S theory where $24\mu+4\mu'\equiv 24\mu+\alpha=1$ we find

$$\begin{aligned} p^{(n+1)}(a_i^2) &= \frac{1-\alpha}{3} p^{(n)}(a_i^2) + \frac{2}{3}(1-\alpha)[p(a_i)]^2 + \alpha p(a_i) \\ p^{(n+1)}(a_ia_k) &= \frac{1-\alpha}{3} p^{(n)}(a_ia_k) + \frac{2}{3}(1-\alpha)p(a_i)p(a_k). \end{aligned} \tag{11}$$

Next consider the case s=3. Computing in the same way as before we find the result:

$$(12)\qquad \begin{aligned} p^{(n+1)}(a_1^3) &= 16\mu_0 p^{(n)}(a_1^3) + 144\mu_1 p^{(n)}(a_1^2)p(a_1) \\ &\quad + 24\mu_0' p^{(n)}(a_1^2) + 36\mu_1'[p(a_1)]^2. \end{aligned}$$

We shall now interpret formula (12) and similar formulas. First let us consider the analogous formula for chromosome segregation (GEIRINGER 1948, p. 259):

$$(12')\qquad \begin{aligned} p^{(n+1)}(A^3) &= \lambda_0 p^{(n)}(A^3) + 9\lambda_1 p(A)p^{(n)}(A^2) \\ &\quad + 9\lambda_2 p^{(n)}(A^2)p(A) + \lambda_3 p^{(n)}(A^3). \end{aligned}$$

We want to analyze it in some detail and consider, for example, the term $9\lambda_2 p^{(n)}(A^2)p(A)$, assuming, for the sake of simplicity, two alleles A and a. Here $p^{(n)}(A^2)=p_{12}^{(n)}(A^2)=p^{(n)}(A^3)+p^{(n)}(A^2a)$ is the probability of a gamete for which the first two of the three genes equal A. In the same way $p_1(A)=p(A)=p^{(n)}(A^3)+p^{(n)}(AAa)+p^{(n)}(AaA)+p^{(n)}(Aaa)$ is the probability of a gamete for which the first of the three genes equals A. We know that $p_{12}^{(n)}(A^2)=p_{13}^{(n)}(A^2)=p_{23}^{(n)}(A^2)=p^{(n)}(A^2)$ and $p_1^{(n)}(A)=p_2^{(n)}(A)=p_3^{(n)}(A)=p^{(n)}(A)=p(A)$. Next consider:

$$\begin{aligned} p^{(n)}(A^2)p(A) &= [p^{(n)}(A^3) + p^{(n)}(A^2a)]\cdot[p^{(n)}(A^3) + 2p^{(n)}(A^2a) + p^{(n)}(Aa)^2)] \\ &= p^{(n)}(A^3)\cdot p^{(n)}(A^3) + p^{(n)}(A^2a)p^{(n)}(A^3) + \cdots \\ &= w^{(n+1)}(A^3; A^3) + w^{(n+1)}(A^2a; A^3) + \cdots + w^{(n+1)}(A^2a; Aa^2) \\ &= \sum_x \sum_y \sum_z w^{(n+1)}(AAx; Ayz). \end{aligned}$$

Consider any of the six $w^{(n+1)}$-terms. For example $9\lambda_2 w^{(n+1)}(A^3; A^3)$ is the probability that, in the $(n+1)^{st}$ generation a genotype be of type $(A^3; A^3)$ and transmits two of its three maternal A-alleles (can happen in three ways) and one of its three paternal alleles (in three ways). In the same way:

$$\begin{aligned} &9\lambda_2 w^{(n+1)}(A^2a;A^3) \\ &\quad = 3\lambda_2[w^{(n+1)}(AAa; A^3) + w^{(n+1)}(AaA; A^3) + w^{(n+1)}(aAA; A^3)]. \end{aligned}$$

Here $3\lambda_2 w^{(n+1)}(AAa; A^3)$ is the probability of this genotype times the probability that it transmits its two maternal A-genes and (in three ways) one of its three paternal A-genes. Hence, on the whole, $9\lambda_2 p^{(n)}(A)p^{(n)}(A)$ *is the probability of a zygote in the* $(n+1)^{st}$ *generation, which possesses at least two maternal and, at least, one paternal A-alleles and which transmits a gamete consisting of two maternal and one paternal A-alleles.* It is clear from this analysis that the sum of the four terms to the right of (12′) gives the probability of a gamete of type (A^3) in the $(n+1)^{st}$ generation.

In case of chromatid segregation the discussion of the terms to the right of (12) which contain μ_0 or μ_1 is exactly the same. Of course $72\mu_1$ plays the role of $9\lambda_1$, etc.

Next consider the "*non-homogeneous*" *terms* in (12) namely

$$36\mu_1'p(A)^2 + 24\mu_0'p^{(n)}(A^2) = 12\mu_3'p^{(n)}(A^2) + 18\mu_2'p(A)^2 + 18\mu_1'p(A)^2 + 12\mu_0'p^{(n)}(A^2).$$

Such terms are due to a double-counting pair of sister chromatids. Consider for instance, $18\mu_2'[p(A)]^2$. Here, as before $[p(A)]^2 = \sum w^{(n+1)}(A\cdot\cdot;\ A\cdot\cdot)$, where the summation is four-fold, the empty places being filled with all combinations of A and a. Consider, as before, one of these terms, for example

$$18\mu_2'w^{(n+1)}(AAA;\ Aaa) = 6\mu_2'[w^{(n+1)}(AAA;\ Aaa) + w^{(n+1)}(AAA;\ aAa) + w^{(n+1)}(AAA;\ aaA)].$$

Here $6\mu_2'w^{(n+1)}(A^3;\ Aaa)$ is the probability of a zygote of type $(A^3;\ Aaa)$ times the probability that this zygote transmits one maternal pair of sister chromatids A (in three ways), and one paternal A-chromatid (in two ways). Hence, on the whole $18\mu_2'p(A)^2$ *is the probability of a zygote of the* $(n+1)^{st}$ *generation which possesses at least one maternal and at least one paternal* A*-chromosome and transmits one pair of maternal sister chromatids,* A, A, *and one paternal* A*-chromatid.*

In an analogous way $p^{(n)}(A^2)\times 1 = \sum w^{(n+1)}(AA\cdot;\ \cdot\cdot\cdot)$ where the empty places are to be filled with the 16 possible combinations of A and a. Consider such a term, as for instance $12\mu_3'w^{(n+1)}(A^2a;\ Aa^2) = 4\mu_3'[w^{(n+1)}(AAa;\ a^2A) + w^{(n+1)}(AaA;\ a^2A) + \cdot]$. Out of the four maternal A-chromatids a triple (containing necessarily a pair of sister chromatids) can be chosen in 4 ways; hence $w^{(n+1)}(AAa;\ a^2A)\cdot 4\mu_3'$ is the probability of this genotype, times the probability that it transmits (in four ways) a maternal triple of A-chromatids. Thus it is seen that the sum of all these "non-homogeneous" terms plus the homogeneous terms to the right of (12) gives just the probability of the gamete (A^3) in the $(n+1)^{st}$ generation (if A stands for a_1).

Analogous considerations hold, of course, for (9).

In addition to (12) we may for $s=3$ derive a recurrence formula for $p^{(n+1)}(a_1^2a_2)$ and $p^{(n+1)}(a_1a_2a_3)$:

$$\begin{aligned} p^{(n+1)}(a_i^3) &= 16\mu_0p^{(n)}(a_i^3) + 144\mu_1p^{(n)}(a_i^2)p(a_i) + 24\mu_0'p^{(n)}(a_i^2) + 36\mu_1'[p(a_i)]^2 \\ p^{(n+1)}(a_i^2a_j) &= 16\mu_0p^{(n)}(a_i^2a_j) + 48\mu_1p^{(n)}(a_i^2)p(a_j) + 96\mu_1p^{(n)}(a_ia_j)p(a_i) + 8\mu_0'p^{(n)}(a_ia_j) + 12\mu_1'p(a_i)p(a_j) \\ p^{(n+1)}(a_ia_ja_k) &= 16\{\mu_0p^{(n)}(a_ia_ja_k) + 3\mu_1[p(a_i)p^{(n)}(a_ja_k) + \cdot + \cdot]\}. \end{aligned} \tag{13}$$

For the interpretation of the terms to the right of (13) similar considerations hold as before.

Upon addition of $p^{(n+1)}(a_1^3)+p^{(n+1)}(a_1^2a_2)+\cdots$ in (13) we get a recurrence formula for $p^{(n+1)}(a_1^2)$, and, in a similar way, one for $p^{(n+1)}(a_1a_2)$. The result is:

$$\begin{aligned} p^{(n+1)}(a_i^2) &= (16\mu_0 + 48\mu_1 + 16\mu_0')p^{(n)}(a_i^2) + (96\mu_1 + 24\mu_1')p(a_i)^2 + (8\mu_0' + 12\mu_1')p(a_i) \\ p^{(n+1)}(a_ia_j) &= (16\mu_0 + 48\mu_1 + 16\mu_0')p^{(n)}(a_ia_j) + (96\mu_1 + 24\mu_1')p(a_i)p(a_j). \end{aligned} \tag{14}$$

Addition of these two formulas shows, as in (10) as anticipated in our notation:

$$p^{(n)}(a_i) = p^{(0)}(a_i) \qquad (n = 1, 2, \cdots ; i = 1, 2, \cdots , r).$$

Again, we may consider particular cases of these formulas by specifying the s.d.:

Random chromatid segregation correspond to $\mu_0=\mu_1=\mu_0'=\mu_1'=\frac{1}{220}$. We have in this case

$$p^{(n+1)}(a_i^3) = \frac{4}{55}p^{(n)}(a_i^3) + \frac{36}{55}p^{(n)}(a_i^2)p(a_i) + \frac{6}{55}p^{(n)}(a_i^2) + \frac{9}{55}p(a_i)^2. \tag{15}$$

For FISHER-MATHER's theory with $\mu_0=\mu_1=\mu$, $\mu_0'=\mu_1'=\mu'$, $60\mu'=\beta$:

$$p^{(n+1)}(a_i^3) = \frac{1-\beta}{10}p^{(n)}(a_i^3) + \frac{9}{10}(1-\beta)p^{(n)}(a_i^2)p(a_i) + \frac{4\beta}{10}p(a_i^2) + \frac{6\beta}{10}p(a_i)^2$$

$$p^{(n+1)}(a_i^2a_j) = \frac{1-\beta}{10}p^{(n)}(a_i^2a_j) + \frac{3(1-\beta)}{10}p^{(n)}(a_i^2)p(a_j) + \frac{6(1-\beta)}{10}p^{(n)}(a_ia_j)p(a_i) + \frac{2\beta}{15}p^{(n)}(a_ia_j) + \frac{3\beta}{15}p(a_i)p(a_j). \tag{16}$$

We shall now finish this section with the *recurrence formulae* for s=4. We find:

$$\begin{aligned} p^{(n+1)}(a_i^4) = {} & 16[2\mu_0p^{(n)}(a_i^4) + 2\cdot 16\mu_1p^{(n)}(a_i^3)p(a_i) + 36\mu_2p(a_i)^2] \\ & + 96[\mu_0'p^{(n)}(a_i^3) + 4\mu_1'p^{(n)}(a_i^2)p(a_i) \\ & + 2\mu_2'p^{(n)}(a_i^2)p(a_i)] + 4[3\mu_0''p^{(n)}(a_i^2) + 4\mu_2''p(a_i)^2]. \end{aligned} \tag{17}$$

To understand the non-homogeneous terms we need (6). Consider for example, $48\mu_4'p^{(n)}(a_i^3)$ or, (with two alleles to fix the ideas) $48\mu_4'p^{(n)}(A^3)\cdot 1 = 48\mu_4'\sum w^{(n+1)}(AAA\cdot, \cdots\cdot)$. Now four A-chromatids belonging to three chromosomes can be selected from eight A-chromatids in $4\cdot 12=48$ ways. Next the term $192\mu_3'p^{(n)}(A^2)p(A)$ corresponds to combining three maternal chromatids from two maternal chromosomes with one paternal chromatid. This can happen in $(4\cdot 6)\cdot 8=192$ ways. The term $96\mu_2'p^{(n)}(A^2)p(A) = (28-4)\cdot 4\mu_2'p^{(n)}(A^2)p(A)$ corresponds to the combination of two maternal chromatids (from two chromosomes) with two paternal sister-chromatids (from one chromosome), or vice versa; etc. etc.

In a similar way we may derive and interpret the formula

$$\begin{aligned} p^{(n+1)}(a_i^3a_j) = {} & 16\{2\mu_0p^{(n)}(a_i^3a_j) + 8\mu_1[p^{(n)}(a_i^3)p(a_j) + 3p^{(n)}(a_i^2a_j)p(a_i)] \\ & + 36\mu_2p^{(n)}(a_i^2)p^{(n)}(a_ia_j)\} \\ & + 48\{\mu_0'p^{(n)}(a_i^2a_j) + 2\mu_1'p^{(n)}(a_i^2)p(a_j) \\ & + (2\mu_1' + 2\mu_2')p^{(n)}(a_ia_j)p(a_i)\}. \end{aligned} \tag{18}$$

AN EXAMPLE

We shall illustrate the recurrence formulas by an example where $s=3$. In order to be able to apply our recurrence formulas we must first derive $p^{(0)}(z)$ from $w^{(0)}(x; y)$ the way it was done in (7) for $s=2$. The formula corresponding to (7), if $s=3$, is:

$$
\begin{aligned}
p(A^3) = {} & v(A^3; A^3)\cdot 1 + 3v(A^3; A^2a)\,[8\mu_0 + 72\mu_1 + 16\mu_0' + 24\mu_1'] \\
& + 3v(A^3; Aa^2)\,[8\mu_0 + 24\mu_1 + 12\mu_0' + 12\mu_1'] \\
& + v(A^3; a^2)(8\mu_0 + 12\mu_0') + 9v(A^2a; A^2a)(16\mu_1 + 4\mu_0' + 8\mu_1') \\
& + 9v(A^2a; Aa^2)(8\mu_1 + 4\mu_0' + 8\mu_1') + 3v(A^2a; a^3)\cdot 4\mu_0' \\
& + 9v(Aa^2; Aa^2)\cdot 4\mu_1'
\end{aligned} \tag{19}
$$

and similar formulas for $p(A\ a)$, etc. In most applications in the given distribution the probabilities of a few types only will be different from zero so that for the derivation of $p^{(0)}(z)$ a general formula like (19) is not needed.

Now consider the *following* simple *example:* A female organism of type $(A^3; A^3)$ is mated to $(a^3; a^3)$. Assuming continued random mating we should like to know the distribution of genotypes in the third filial generation if FISHER's theory of four strand segregation is considered. According to the data we have

$$
\begin{aligned}
& v^{(0)}(A^3; A^3) = 1 \text{ (females)}, \qquad \bar{v}^{(1)}(a^3; a^3) = 1 \text{ (males)} \\
& p^{(0)}(A^3) = 1, \text{ all others zero}, \qquad \bar{p}^{(1)}(a^3) = 1 \text{ (all others zero)}.
\end{aligned}
$$

In the next generation, for males and for females: $v^{(1)}(A^3; a^3) = 1$, all others zero.

It follows that

$$
\begin{aligned}
p^{(1)}(A^3) &= v^{(1)}(A^3; a^3)(8\mu_0 + 12\mu_0') = 8\mu_0 + 12\mu_0' \\
3p^{(1)}(A^2a) &= v^{(1)}(A^3; a^3)(72\mu_2 + 18\mu_2') = 72\mu_1 + 18\mu_1' \\
3p^{(1)}(Aa^2) &= v^{(1)}(A^3; a^3)(72\mu_1 + 18\mu_1') = 72\mu_1 + 18\mu_1' \\
p^{(1)}(Aa^3) &= v^{(1)}(A^3; a^3)(8\mu_0 + 12\mu_0 P') = 8\mu_0 + 12\mu_0'.
\end{aligned}
$$

Now with FISHER-MATHER's assumptions:

$$
\begin{aligned}
p^{(1)}(A^3) = p^{(1)}(a^3) &= \frac{1-\beta}{20} + \frac{\beta}{5} = \frac{1+3\beta}{20} \\
3p^{(1)}(A^2a) = 3p^{(1)}(Aa^2) &= \frac{9}{20}(1-\beta) + \frac{3\beta}{20} = \frac{9-3\beta}{20} \\
p^{(1)}(A^2) = p^{(1)}(a^2) &= \frac{2+\beta}{10} \\
p^{(1)}(Aa) &= \frac{3-\beta}{10} \\
p^{(1)}(A) = p^{(1)}(a) &= \frac{1}{2}\,.
\end{aligned}
$$

Next we use the recurrence formulas and find:

$$p^{(2)}(A^3) = \frac{1-\beta}{10}p^{(1)}(A^3) + \frac{9}{10}(1-\beta)p^{(1)}(A^2)p(A) + \frac{4\beta}{10}p^{(1)}(A^2) + \frac{6\beta}{10}p(A)^2$$

$$= \frac{1-\beta}{10}\,\frac{1+3\beta}{20} + \frac{9}{10}(1-\beta)\,\frac{2+\beta}{10}\cdot\frac{1}{2} + \frac{4\beta}{10}\,\frac{2+\beta}{10} + \frac{6\beta}{10}\cdot\frac{1}{4}$$

$$= \frac{19+39\beta-4\beta^2}{200} = p^{(2)}(a^3),$$

$$p^{(2)}(A^2a) = \frac{1-\beta}{10}p^{(1)}(A^2a) = \frac{3(1-\beta)}{10}p^{(1)}(A^2)p(a) + \frac{6(1-\beta)}{10}\,p^{(1)}(Aa)\cdot\frac{1}{2}$$

$$+ \frac{2\beta}{15}p^{(1)}(Aa) + \frac{3\beta}{15}p(A)p(a)$$

$$= \frac{(1-\beta)(3-\beta)}{200} + \frac{3(1-\beta)(2+\beta)}{200} + \frac{6(1-\beta)(3-\beta)}{200}$$

$$+ \frac{2\beta}{15}\,\frac{3-\beta}{10} + \frac{3\beta}{15}\cdot\frac{1}{4}$$

$$= \frac{1}{3}\,\frac{81-39\beta+4\beta^2}{200} = p^{(2)}(Aa^2).$$

Also:

$$p^{(2)}(A^2) = p^{(2)}(a^2) = \frac{69+39\beta-4\beta^2}{300}$$

$$p^{(2)}(Aa) = \frac{81-39\beta+4\beta^2}{300}.$$

In order to estimate the order of magnitude of the various terms introduce $\beta = 0.117$ (FISHER and MATHER 1943, p. 17). We find:

$$\frac{19+39\beta-4\beta^2}{200} = .118 \qquad \frac{81-39\beta+4\beta^2}{200} = .382$$

and finally by (8) for the third generation:

$$v^{(3)}(A^3; A^3) = v^{(3)}(a^3; a^3) = \tfrac{1}{2}v^{(3)}(A^3; a^3) = .014$$

$$3v(A^3; A^2a) = 3v(A^3; Aa^2) = 3v(a^3; A^2a)$$

$$= 3v(a^3; Aa^2) = .090$$

$$9v(A^2a; A^2a) = 9v(Aa^2; Aa^2) = \tfrac{1}{2}\cdot 9v(A^2a; Aa^2) = .146$$

and

$$4\cdot(.014 + .090 + .146) = 1.000.$$

INTEGRATION OF THE RECURRENCE FORMULAS AND LIMIT THEOREMS

We start integrating the formulas for $s=2$. We find immediately:

$$
\begin{aligned}
p^{(n)}(a_i^2) &= (8\mu_0)^n p^{(0)}(a_i^2) \\
&\quad + [1-(8\mu_0)^n]\cdot\left[\frac{4\mu_1}{4\mu_1+\mu_0'}\,p(a_i)^2 + \frac{\mu_0'}{4\mu_1+\mu_0}\,p(a_i)^2\right] \\
p^{(n)}(a_ia_k) &= (8\mu_0)^n p^{(0)}(a_ia_k) \\
&\quad + [1-(8\mu_0)^n]\,\frac{4\mu_1}{4\mu_1+\mu_0'}\,p(a_i)p(a_k)
\end{aligned}
\tag{20}
$$

or, in FISHER's theory:

$$
\begin{aligned}
p^{(n)}(a_i^2) &= \left(\frac{1-\alpha}{3}\right)^n p^{(0)}(a_i^2) \\
&\quad + \left[1-\left(\frac{1-\alpha}{3}\right)^n\right]\cdot\left[\frac{2-2\alpha}{2+\alpha}\,p(a_i)^2 + \frac{3\alpha}{2+\alpha}\,p(a_i)\right] \\
p^{(n)}(a_ia_k) &= \left(\frac{1-\alpha}{3}\right)^n p^{(0)}(a_ia_k) \\
&\quad + \left[1-\left(\frac{1-\alpha}{3}\right)^n\right]\frac{2-2\alpha}{2+\alpha}\,p(a_i)p(a_k).
\end{aligned}
\tag{21}
$$

We see from these formula that the *rate of approach to equilibrium* is $(8\mu_0)^n$ *or* $(1-\alpha/3)^n$ *respectively*$_n$ it is $(\frac{2}{7})^n$ *for random chromatid segregation,* $(\frac{5}{16})^n$ *for complete equational* and $(\frac{1}{3})^n$ *for chromosome segregation.* These figures differ but little from each other.

From (20) we find, *if* $8\mu_0<1$, *the limit formulas*:

$$
\begin{aligned}
\lim_{n\to\infty} p^{(n)}(a_i)^2 &= \frac{4\mu_1}{4\mu_1+\mu_0'}\,p(a_i)^2 + \frac{\mu_0'}{4\mu_1+\mu_0}\,p(a_i) \\
\lim_{n\to\infty} p^{(n)}(a_ia_k) &= \frac{4\mu_1}{4\mu_1+\mu_0'}\,p(a_i)p(a_k)
\end{aligned}
\tag{22}
$$

If, however, $8\mu_0=1$, that is, $\mu_1=\mu_0'=0$, we see that (see Geiringer 1948, p. 277)

$$
p^{(n)}(a_i^2) = p^{(0)}(a_i^2), \quad p^{(n)}(a_ia_k) = p^{(0)}(a_ia_k), \qquad (n = 1, 2, \cdots). \tag{22'}
$$

Under FISHER's *assumptions* we get the limit formula:

$$
\begin{aligned}
\lim_{n\to\infty} p^{(n)}(a_i^2) &= \frac{2-2\alpha}{2+\alpha}\,p(a_i)^2 + \frac{3\alpha}{2+\alpha}\,p(a_i) \\
\lim_{n\to\infty} p^{(n)}(a_ia_k) &= \frac{2-2\alpha}{2+\alpha}\,p(a_i)p(a_k)
\end{aligned}
\tag{23}
$$

and for *random chromatid segregation*

$$\lim_{n\to\infty} p^{(n)}(a_i^2) = \frac{4}{5}\,p(a_i)^2 + \frac{1}{5}\,p(a_i)$$
$$\lim_{n\to\infty} p^{(n)}(a_i a_k) = \frac{4}{5}\,p(a_i)p(a_k). \tag{24}$$

The results (24), for $s=2$, have been given by HALDANE (1930, p. 370). The recurrence formulas (9''), the explicit solutions (20), (21) as well as the limit results (22), (23) are new. All results are new in the case $s=3$, which we now shall consider.

We have already found the recurrence formulas (13) and (14). Here (13) can be integrated too. First we find the explicit result for $p^{(n)}(a_i^2)$ which corresponds to (14) and is of form (20). Introducing this into the right side of the first formula (13) this right side becomes a known function, f_n, of n. Denoting $p^{(n)}(a_i^3)$ by x_n and $16\mu_0 = a$, we have the equation $x_{n+1} - a x_n = f_n$ which has the solution

$$x_n = a^n x_0 + \sum_{\gamma=0}^{n-1} f_\gamma a^{n-1-\gamma}.$$

This can be computed for every given s.d.

Let us consider the limit of the $p^{(n)}$ as $n\to\infty$. Assume $16\mu_0 < 1$. It then follows that in (14), $16\mu_0 + 48\mu_1 + 16\mu_0' < 1$. If this would not be so we could conclude that $\mu_1 = \mu_1' = \mu_0' = 0$ hence $16\mu_0 = 1$. We get from (14)

$$\lim_{n\to\infty} p^{(n)}(a_i^2) = \frac{(24\mu_1 + 6\mu_1')p(a_i)^2 + (2\mu_0' + 3\mu_1')p(a_i)}{24\mu_1 + 9\mu_1' + 2\mu_0'}. \tag{25}$$

Hence in (13) which is of the form $x_{n+1} - a x_n = f_n$ we know the $\lim_{n\to\infty} f_n = f$. It follows from a lemma (GEIRINGER 1948, p. 262) that, if

$$|a| < 1, \qquad \lim x_n = \frac{f}{1-a}.$$

In our case we have:

$$f = [144\mu_1 p(a_i) + 24\mu_0']\cdot\frac{(24\mu_1 + 6\mu_1')p(a_i)^2 + (2\mu_0' + 3\mu_1')p(a_i)}{24\mu_1 + 9\mu_1' + 2\mu_0'} + 36\mu_1' p(a_i)^2.$$

Our result is, if

$16\mu_0 < 1$ and $(12\mu_1 + 3\mu_1' + 2\mu_0')(24\mu_1 + 9\mu_1' + 2\mu_0') = M$:

$$\lim_{n\to\infty} p^{(n)}(a_i^3) = \frac{1}{M}\cdot\{(288\mu_1^2 + 72\mu_1\mu_1')p(a_i)^3 + (72\mu_1\mu_0' + 108\mu_1\mu_1' + 18\mu_0'\mu_1' + 27\mu_1'^2)p(a_i)^2 + (4\mu_0'^2 + 6\mu_0'\mu_1')p(a_i)\}. \tag{26}$$

If $16\mu_0 = 1$:

$$p^{(n)}(a_i^3) = p^{(0)}(a_i^3).$$

In case of ***random chromatid segregation*** (26) gives:

$$\lim_{n\to\infty} p^{(n)}(a_i^3) = \frac{72}{7\cdot 17}\, p(a_i)^3 + \frac{45}{7\cdot 17}\, p(a_i)^2 + \frac{2}{7\cdot 17}\, p(a_i). \tag{27}$$

We may derive similar results for $p^{(n)}(a_i^2 a_k)$. First we have a second formula (25):

$$\lim_{n\to\infty} p^{(n)}(a_i a_k) = \frac{24\mu_1 + 6\mu_1'}{24\mu_1 + 2\mu_0' + 9\mu_1'}\, p(a_i)p(a_k). \tag{25}$$

Now we proceed with the second formula (13) exactly as with the first. In fact, if we substitute on the right side of this formula the values (14) for $p^{(n)}(a_i^2)$ and $p^{(n)}(a_i a_k)$ this right side becomes a known function of n, say z_n, such that $\lim_{n\to\infty} z_n = z$ exists and

$$\lim_{n\to\infty} p^{(n)}(a_i^2 a_k) = \frac{z}{1 - 16\mu_0}.$$

The result is:

$$\begin{aligned} \lim_{n\to\infty} p^{(n)}(a_i^2 a_k) &= \frac{1}{M}\cdot\{(288\mu_1^2 + 72\mu_1\mu_1')p(a_i^2)p(a_k) \\ &\quad + (24\mu_1\mu_0' + 36\mu_1\mu_1' + 6\mu_1'\mu_0' + 9\mu_1'^2)p(a_i)p(a_k)\}, \\ \lim_{n\to\infty} p^{(n)}(a_i a_j a_k) &= \frac{1}{M}\, 12\mu_1(24\mu_1 + 6\mu_1')p(a_i)p(a_j)p(a_k). \end{aligned} \tag{26}$$

For random chromatid segregation this becomes:

$$\begin{aligned} \lim_{n\to\infty} p^{(n)}(a_i^2 a_k) &= \frac{72}{7\cdot 17}\, p(a_i^2)p(a_k) + \frac{15}{7\cdot 17}\, p(a_i)p(a_k), \\ \lim_{n\to\infty} p^{(n)}(a_i a_j a_k) &= \frac{72}{7\cdot 17}\, p(a_i)p(a_j)p(a_k). \end{aligned} \tag{27}$$

Finally, with FISHER's and MATHER's assumption; where

$160\mu + 60\mu' = 160\mu + \beta = 1$:

$$\begin{aligned} \lim_{n\to\infty} p^{(n)}(a_i^3) &= \frac{27(1-\beta)(3-\beta)}{(9+\beta)(9+2\beta)}\, p(a_i)^3 + \frac{45\beta(3-\beta)}{(9+\beta)(9+2\beta)}\, p(a_i)^2 \\ &\quad + \frac{20\beta^2}{(9+\beta)(9+2\beta)}\, p(a_i), \\ \lim_{n\to\infty} p^{(n)}(a_i^2 a_k) &= \frac{27(1-\beta)(3-\beta)}{(9+\beta)(9+2\beta)}\, p(a_i)^2 p(a_k) \end{aligned} \tag{28}$$

$$+\frac{15\beta(3-\beta)}{(9+\beta)(9+2\beta)}\,p(a_i)p(a_k),$$

$$\lim_{n\to\infty} p^{(n)}(a_ia_ja_k) = \frac{27(1-\beta)(3-\beta)}{(9+\beta)(9+2\beta)}\,p(a_i)p(a_j)p(a_k).$$

The *rate of approach to equilibrium* is $(16\mu_0+48\mu_1+16\mu_0')^n$ This is $\left(\frac{6-2\beta}{15}\right)^n$ under FISHER'S assumption, and $\left(\frac{4}{11}\right)^n$ for random chromatid segregation, compared to $\left(\frac{4}{10}\right)^n$ for random chromosome segregation.

In order to compute the limit status for the example in the preceding section we merely have to put in (28): $p(a_1)=p(a_2)=\frac{1}{2}$, $\beta=.1176$.

Similar results may be established for $s=4$, using (17) and (18).

We see that the limit results under the assumption of chromatid segregation are essentially different from the ones for chromosome segregation where, in the limit, the alleles were independently distributed. By multiplication of the limit expressions (26) or (27) or (28) we get the respective limit-distributions of genotypes.

SUMMARY

The mathematical genetics of autopolyploids under chromatid segregation, is dealt with, in particular for tetraploids, hexaploids, and octoploids. The segregation-distribution introduced by R. A. FISHER and K. MATHER [1943] has been generalized so as to contain as particular cases this segregation distribution as well as HALDANE'S "random chromatid segregation" [1930] and the author's [1948] general chromosome segregation distribution. The possible kinds of gametes and of genotypes are enumerated, recurrence relations for their distributions are established and "integrated," and limit results as, n, the number of discrete non-overlapping generations tends to infinity, are investigated. The recurrence relations as well as the limit theorems are essentially different from the analogous results under chromosome segregation and seem to present a new type of statistical-biological law.

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TETRAPLOID RATIOS OF SELF-STERILITY ALLELES: A CORRECTION

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IN a preliminary account of the induced tetraploids of a self-sterile race of *Oenothera rhombipetala* the writer (HECHT 1944) calculated the expected gametic ratios upon a basis of preferential pairing of chromosomes dissimilar with respect to their self-sterility alleles (allosyndesis). Upon this basis a 1:2:1 ratio of homozygote:heterozygote:homozygote was reported. LINDSTROM (1936) has pointed out that there is no reason to expect selective allosyndesis, and that data on the segregation of autopolyploids do not conform to the ratios which would be expected under such a mechanism. If, on the other hand, there were preferential pairing of similar chromosomes (autosyndesis) each reciprocal cross of the first generation autotetraploids of *O. rhombipetala* would have given but a single class of progeny instead of the three actually obtained. Eliminating these two types of selective pairing, there remains the possibility of random association. If the chromosomes were associated as bivalents, random assortment would result in a 1:4:1 gametic ratio of homozygote:heterozygote:homozygote. This same ratio would be expected with quadrivalent association only if the locus of the gene were so close to the centromere that crossing over between the locus and the centromere does not occur. If crossing over does occur in this portion of the chromosome, the gametic ratio which would result is affected by the many factors which affect the frequency and type of crossing over. MATHER (1935; 1936) has analyzed the complexities of segregation under these circumstances, and the entire situation has more recently been reviewed by LITTLE (1945). Random assortment of the eight chromatids would result in a 3:8:3 ratio of the gametic types, but MATHER has shown that random assortment probably never occurs, and that the degree to which it is approached is almost impossible to analyze. Consequently definite theoretical ratios cannot be assigned in such instances. Since autotetraploid *Oenothera rhombipetala* does form quadrivalents its segregation is subject to these complications.

In going over these data the writer has uncovered the following additional errata: page 72, line 16 (2nd line below fig. 2) YX should read XY; page 72, delete beginning "Plant 18 of YX" (line 20) to end of paragraph, and substitute the following: Plant 2 of XY can be class B or C, but not class A, for it fails to set seed with pollen of YX 20 (B′). Since YX 18 does set seed when pollinated with XY 2 (B or C), the seed parent must be class A′. XY 2 cannot be C, for it fails to set seed when pollinated with YX 18 (A′); therefore it must be class B.

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THE GENETIC ANALYSIS OF TWO CHROMOSOME INTERCHANGES IN BARLEY FROM F_2 DATA[1]

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THE use of backcross data for the genetic analysis involving an interchange between two non-homologous chromosomes has been clearly defined and reviewed. JOACHIM (1947) has presented tables to facilitate the use of the product method for the analysis of F_2 data involving semisterility. In a self-pollinated plant such as barley, this method presents a convenient tool for the determination of linkage intensities between semisterility and a qualitative factor pair when there are four F_2 phenotypic classes. The recombination value thus obtained has the same standard error as that obtained by the method of maximum likelihood. The object of this paper is to present the genetic analysis of two chromosomal interchanges in barley from F_2 data.

The term semisterility was originally used to describe individuals showing about 50 percent pollen sterility. When explained by the chromosomal interchange theory, semisterility became a convenient designation for those individuals heterozygous for an interchange. This connotation will be followed in this paper although in barley the interchange causes only about 25 percent sterility.

MATERIALS AND METHODS

Reciprocal translocations as they occur in nature can be duplicated by exposing to high frequency radiation a meristematic tissue which is predetermined to bear reproductive structures. SMITH (1939) outlined a procedure for the X-ray treatment of *Triticum monococcum* and *T. Aegilopoides* seeds for the inducement of chromosomal abnormalities. The writers are indebted to DR. C. R. BURNHAM of the Division of Agronomy and Plant Genetics, UNIVERSITY OF MINNESOTA for the materials used in this study. The analysis of the interchanges C-1420 and C-1432, referred to as Purdue Accessions 300 and 301 respectively, will be presented in this study.

The linkage group to which each character belongs based on the summary of linkage studies in barley by ROBERTSON *et al.* (1947), the genetic constitution of the interchange parents, and the tester stocks used to study the linkage between semisterility and the factors are summarized in table 1.

During the summer of 1947, crosses were made in the field between the interchange stocks and the five genetic testers. F_1 individuals were grown in the greenhouse during the winter of 1947–48. Poor germination resulting from dormancy necessitated planting all of the F_2 seeds in drilled rows in the field

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in 1948, the F_2 plants later being thinned randomly to a spaced-planting of 3 inches. Crosses between an interchange line and a genetic tester were grown in F_2 family rows. These rows served as a check against possible greenhouse misclassification and, in the crosses involving a tester line which was segregating for a lethal seedling character, permitted recognition of homozygous and heterozygous plants in the tester line.

The classification for semisterility was made on the basis of ovule fertility at the time the plants were threshed. The justification for this classification

TABLE 1

Summary of factor pairs studied together with the linkage group to which each factor pair belongs, the genotype of the interchange, and the genetic stock used to study the linkage.

LINKAGE GROUP	CHARACTER	SYMBOLS	GENOTYPE INTERCHANGE	TESTER USED TO STUDY LINKAGE
I	Two vs. six-row	*V, v*	*vv*	293
II	Black vs. white pericarp and lemma	*B, b*	*bb*	293
III	Covered vs. naked caryopsis	*N, n*	*NN*	293
IV	Hooded vs. awned	*K, k*	*kk*	293, 294, 295
	Blue vs. non-blue aleurone	*Bl, bl*	*blbl*	296
V	Long vs. short-haired rachilla	*S, s*	*SS*	294, 295. 296
	Rough vs. smooth awns	*R, r*	*rr*	296
VI	Green vs. xantha seedlings	*Xc, xc*	*XcXc*	294 seg
VII	Starchy vs. waxy endosperm*	*Wx, wx*	*WxWx*	297
	Green vs. virescent seedlings	*Yc, yc*	*YcYc*	296 seg
	Green vs. chlorina seedlings	*Fc, fc*	*FcFc*	295
Unknown	Green vs. light green seedlings**	Lg_2, lg_2	Lg_2Lg_2	293 seg

* Unpublished data of O. J. WEBSTER (personal correspondence), G. A. WIEBE and R. G. SHANDS place *Wx, wx* in the VII linkage group.

** The homozygous recessive $lg_2\,lg_2$ produced a light yellow-green seedling which proved to be a lethal seedling character.

for semisterility would depend upon the ability to resolve the ovule sterility due to a chromosome interchange from that which is a function of the environment. From a quantitative study involving interchanges, data were available to study this interaction. The ovule sterility of the interchange parents and of the tester parents ranged from 1 percent to 13 percent with the modal class being the class interval 1 percent to 2 percent. The frequency distribution curve of the ovule sterility for the F_2 progeny rows was bimodal with the maximum class intervals being 1 percent to 2 percent and 23 percent to 24 percent respectively. From a study of these frequency distributions, 12 percent was selected as the point of differentiation between normal and semisterile individuals.

As a precaution, a head was clipped and tagged from each plant at the time of anthesis, these heads being fixed in a solution of 3 parts alcohol and 1 part glacial acetic acid. A critical comparison between these two methods of classification was made. Based on these comparisons, the conclusion that ovule

classification for semisterility could be used in preference to microscopic examination of the pollen seemed warranted. Errors in pollen classification arise through poor sources of pollen such as immature or old pollen; the accuracy of ovule classification is affected by environmental influences. Errors in ovule classification can be reduced by individually-spaced plantings. In addition, ovule classification can be done rapidly in contrast to pollen examination, thus permitting the use of larger populations in this study.

PRESENTATION OF DATA

The segregation of semisterile (SS): normal (N) individuals is a very close fit to the expected 1:1 ratio as is shown in table 2. There is a uniformly good fit in all populations.

TABLE 2

The χ^2 test for homogeneity in the segregation of semisterile versus normal individuals.

SOURCE OF INFORMATION	SS	N	χ^2	df
293×300*	68	68	0.00	1
294×300*	80	83	.06	1
295×300	159	181	1.42	1
296×300*	103	99	.08	1
297×300	117	127	.41	1
293×301*	59	50	.74	1
294×301*	109	108	.01	1
295×301	62	69	.37	1
296×301*	157	129	2.74	1
297×301	168	166	.01	1
Total χ^2			5.84	10
Total	1082	1080	.00	1

* Only the population which was not segregating for a lethal seedling was used.

Linkage Groups Involved in Interchange 300

The results of the crosses of interchange 300 with each of the linkage testers are summarized in table 3. The nonsignificant χ^2 values for independence indicate that semisterility is inherited independently from the following marker genes: covered vs. naked caryopsis (*N*, *n*), rough vs. smooth awns (*R*, *r*), green vs. xantha seedlings (*Xc*, *xc*), long vs. short-haired rachilla (*S*, *s*), green vs. virescent seedlings (*Yc*, *yc*), and starchy vs. waxy endosperm (*Wx*, *wx*). Linkage groups 3, 5, 6, and 7 are not involved in this interchange. The tests of the hypothesis that hooded vs. awned (*K*, *k*) and blue vs. non-blue aleurone (*Bl*, *bl*) are inherited independently from semisterility are highly significant indicating linkage of semisterility to genes in the IV linkage group. The linkage relationship of two vs. six-rowed (*V*, *v*) and semisterility is less apparent. The original parent 293 was segregating for a lethal seedling factor Lg_3, lg_3 which

TABLE 3

Summary of tests for linkage between interchange 300 and marker genes.

CROSS	CHARACTER *Aa*	*A*SS	*A*N	*a*SS	*a*N	TOTAL	χ^2 FOR FACTOR Aa†	χ^2 FOR INDEPENDENCE
293×300	Nonsegregating Lg_3							
	Vv	57	51	11	17	136	1.41	1.62
	Bb	58	46	10	22	136	0.16	5.89*
	Nn	51	50	17	18	136	0.04	0.02
	Kk	64	46	4	22	136	2.51	15.41**
293×300	Segregating Lg_3							
	Vv	104	19	18	41	182	5.34*	67.20**
	Bb	87	47	35	13	182	0.18	1.02
	Nn	89	45	33	15	182	0.18	0.09
	Kk	117	7	5	53	182	4.49*	131.44**
	Lg_3lg_3	122	60	0	0	182	21.16**	
294×300	Nonsegregating *Xc*							
	Kk	74	45	6	38	163	0.35	30.81**
	Ss	59	64	21	19	163	0.02	0.25
294×300	Segregating *Xc*							
	Kk	113	63	9	60	245	0.17	51.90**
	Ss	96	87	26	36	245	0.01	2.05
	Xcxc	122	123	0	0	245	0.01	
295×300								
	Kk	151	85	8	96	340	5.66*	91.88**
	Ss	112	138	47	43	340	0.39	1.46
296×300	Nonsegregating *Yc*							
	Rr	76	70	27	29	202	0.80	0.24
	Blbl	89	67	14	32	202	0.53	10.07**
296×300	Segregating *Yc*							
	Rr	65	71	23	12	171	1.87	3.58
	Blbl	79	58	9	25	171	2.39	10.61**
	Ycyc	88	83	0	0	171	0.15	
297×300								
	Wxwx	82	98	35	29	244	0.20	1.58

† Goodness of fit to 3:1 or 1:1 ratios.
* Significant at the 5 percent point.
** Significant at the 1 percent point.

was the basis for the two subpopulations of the cross 293×300. In the nonsegregating populations, the factor pair *V*, *v* is apparently inherited independently from semisterility; however, the deviation is in the direction expected if linkage did exist. Black vs. white pericarp and lemma (*B*, *b*) is apparently

linked to semisterility in this group as indicated by a significant χ^2 test for independence. On the other hand, a highly significant χ^2 test for independence is found between the factor pair *V*, *v* and semisterility but not for the factor pair *B*, *b* and semisterility in segregating Lg_3 populations of 293×300. The linkage between the *V*, *v* locus and semisterility seems evident from the following considerations.

It is apparent from table 3 that semisterility is closely linked to the Lg_3 locus. A segregation of 122 SSLg_3 to 60 NLg_3 occurred which is a significant deviation from the expected 1:1 ratio. As the strength of the linkage between the chromosome break and the locus of a lethal seedling factor increases, the expected ratio of semisterile to normal individuals will approach 2:1 as a limit. If the assumption is made that there is zero recombination between semisterility and the Lg_3 locus, then the following ratios would be expected:

If *V*, *v* independently inherited:

	Nonsegregating Lg_3			Segregating Lg_3	
	V	*v*		*V*	*v*
SS	3	1	SS	6	2
N	3	1	N	3	1

If 0 percent recombination between *V*, *v* and SS(Lg_3):

	Nonsegregating Lg_3			Segregating Lg_3	
	V	*v*		*V*	*v*
SS	2	0	SS	2	0
N	1	1	N	0	1

Thus, the group in which Lg_3 is segregating becomes a sensitive test for linkage. This is very evident in the comparison of χ^2 values for independence between hooded *vs.* awned (*K*, *k*) and semisterility.

Additional evidence to support the hypothesis that linkage exists between the *V*, *v* locus and semisterility is found in the significant deviation of both factor pairs *V*, *v* and *K*, *k* from the expected 3:1 ratio in the segregating population of 293×300. As the strength of the linkage between a character and a lethal seedling factor increases, deviation from the expected 3:1 toward a 2:1 ratio as a limit would be expected. One of these two factor pairs (*V*, *v* or *K*, *k*) is linked directly to Lg_3; the other is demonstrating pseudolinkage to Lg_3. A normal segregation of black vs. white pericarp and lemma is found in this group (table 3).

The final evidence which can be found in these data would be the test for pseudolinkage between the *K*, *k* locus which is known to be linked to semisterility, and the factor pairs *V*, *v* and *B*, *b* respectively. This is true since the two interchange chromosomes and the two corresponding normal chromosomes function as single units during metaphase I of meiotic division. It is evident from table 4 that the factor pairs *V*, *v* and *K*, *k* are not inherited independently in this cross while *B*, *b* and *K*, *k* are. In the cross of 293×301, *K*, *k* is inherited independently from *V*, *v* and *B*, *b* respectively. Linkage groups I and IV must be involved in interchange 300.

TABLE 4

Tests for the existence of pseudolinkage between hooded vs. awned (K, k) and the characters two vs. six-rowed (V, v) and black vs. white pericarp and lemma (B, b), respectively.

CROSS	CHARACTER *Aa*	*AK*	*Ak*	*aK*	*ak*	TOTAL	χ^2 INDEPENDENCE
293×300	Nonsegregating Lg_3						
	Vv	95	13	15	13	136	17.02**
	Bb	87	17	23	9	136	2.20
293×300	Segregating Lg_3						
	Vv	101	22	23	36	182	34.16**
	Bb	90	44	34	14	182	0.22
293×301	Nonsegregating Lg_3						
	Vv	67	17	17	8	109	1.51
	Bb	63	19	21	6	109	0.01
293×301	Segregating Lg_3						
	Vv	74	40	20	9	143	0.17
	Bb	70	34	24	15	143	0.48

** Significant at the 1 percent point.

Linkage Groups Involved in Interchange 301

The results of the crosses of interchange 301 with the linkage testers are summarized in table 5. Independent assortment was found between semisterility and two vs. six-rowed (*V*, *v*), black vs. white pericarp and lemma (*B*, *b*), covered vs. naked caryopsis (*N*, *n*), long vs. short-haired rachilla (*S*, *s*), green vs. xantha seedlings (*Xc*, *xc*), starchy vs. waxy endosperm (*Wx*, *wx*), and green vs. virescent seedlings (*Yc*, *yc*). Linkage apparently exists between semisterility and hooded vs. awned (*K*, *k*) and blue vs. non-blue aleurone (*Bl*, *bl*) respectively. Both *K*, *k* and *Bl*, *bl* are in the IV linkage group. Until additional information is available, only circumstantial evidence can be given for the second linkage group involved in this interchange.

The only direct test with F_2 data for linkage between an interchange and a lethal seedling factor is the χ^2 test for fit to a 1:1 ratio. The deviation of SS*Xc* and N*Xc* from the expected 1:1 ratio is not significant; however, the proportion of semisteriles in this population (105/89) is in the direction expected if linkage exists. Four family rows were combined to obtain the total population. In all of these rows there was a consistent trend toward a predominance of semisterile individuals. Since the factor pair *K*, *k* is linked to semisterility, it should also demonstrate pseudolinkage to xantha in linkage group VI. As is evident from table 5, this deviation from the expected ratio is almost significant (P = ca. 0.07) with the proportion of awned individuals approaching $\frac{1}{3}$ of the population. Since there is no sensitive test for the presence of linkage, additional information from F_3 rows is essential to confirm or to disprove this assumption.

TABLE 5

Summary of tests for linkage between interchange 301 and marker genes.

CROSS	CHARACTER *Aa*	*A*SS	*A*N	*a*SS	*a*N	TOTAL	χ^2 FOR FACTOR Aa†	χ^2 FOR INDEPENDENCE
293×301	Nonsegregating Lg_2							
	Vv	47	37	12	13	109	0.25	0.49
	Bb	47	35	12	15	109	0.00	1.36
	Nn	45	41	14	9	109	0.88	0.53
	Kk	50	34	9	16	109	0.25	4.29*
293×301	Segregating Lg_2							
	Vv	75	39	21	8	143	1.70	0.46
	Bb	70	34	26	13	143	0.39	0.00
	Nn	76	37	20	10	143	1.23	0.00
	Kk	83	11	13	36	143	6.55*	55.69**
	Lg_2lg_2	96	47	0	0	143	16.79**	
294×301	Nonsegregating *Xc*							
	Kk	101	61	8	47	217	0.01	37.53**
	Ss	81	86	28	22	217	0.44	0.87
294×301	Segregating *Xc*							
	Kk	100	35	5	54	194	3.03	81.16**
	Ss	78	63	27	26	194	0.56	0.30
	Xcxc	105	89	0	0	194	1.32	
295×301								
	Kk	56	45	6	24	131	0.31	11.66**
	Ss	47	51	15	18	131	0.00	0.06
296×301	Nonsegregating *Yc*							
	Blbl	138	84	19	45	286	1.05	21.13**
	Rr	119	100	38	29	286	0.38	0.12
296×301	Segregating *Yc*							
	Blbl	35	27	9	19	90	1.79	4.56*
	Rr	32	34	12	12	90	0.13	0.02
	Ycyc	44	46	0	0	90	0.04	
297×301								
	Wxwx	123	122	45	44	334	0.48	0.00

† Goodness of fit to 3:1 or 1:1 ratios.
* Significant at the 5 percent point.
** Significant at the 1 percent point.

The Calculation of Linkage Intensities in the Crosses of Interchanges 300 and 301 to the Genetic Testers

In the crosses of interchange 300 to the genetic testers, linkage exists between semisterility and factors in the I and IV linkage groups respectively,

while the interchange break 301 is linked to factors in linkage groups IV and possibly VI. The sources of information which can be applied to the calculation of a specific recombination value are summarized in table 1. In crosses to a parent which was segregating for a lethal seedling factor and where this factor is linked to semisterility, these sources of information can not be used in the calculation of linkage intensities by the product method. χ^2 tests for population homogeneity were made for these combinable sources of information based on the hypothesis that the total observed ratio was the best estimate of the true ratio. Based upon the nonsignificant χ^2 tests for population homogeneity, the respective sources of data were combined. These combined observed class numbers together with the calculated recombination percentage and the standard error as computed by the tables presented by JOACHIM (1947) are given in table 6.

TABLE 6

Summary of linkage relationships of interchanges 300 and 301 together with the observed numbers and standard error of p.

SOURCE OF INFORMATION	OBSERVED CLASS NUMBERS				TOTAL	p ± SE_p
	a	b	c	d		
Interchange 300						
SS:N vs. *V:v*	57	51	11	17	136	27.5± 8.12
SS:N vs. *K:k*	402	239	27	216	884	6.0± 1.13
SS:N vs. *Bl:bl*	168	125	23	57	373	17.3± 3.17
SS:N vs. Lg_3:lg_3	122	60	0	0	182	.0± 5.24*
V:v vs. *K:k*	95	13	15	13	136	26.8± 4.57
Interchange 301						
SS:N vs. *K:k*	207	140	23	87	457	11.9± 2.25
SS:N vs. *Bl:bl*	173	111	28	64	376	16.5± 5.91
SS:N vs. *Xc:xc*	105	89	0	0	194	25.1±10.80
SS:N vs. Lg_3:lg_3	96	47	0	0	143	.0± 5.91*

* The calculated value for p was negative, zero being accepted as the best estimate.

In the crosses of interchanges 300 and 301 to the genetic tester 293 and in the cross of interchange 301 to the genetic tester 294 linkage exists or is suspected to exist between the break and a lethal seedling factor. An estimate of the intensity of this linkage can be made by applying the method of maximum likelihood to these data. JOACHIM (1947) p. 581 has given the expected F_2 phenotypic class frequencies for linkage involving semisterility and a qualitative factor pair. These class frequencies adjusted (see KRAMER and BURNHAM 1947) to a total class frequency of 1 are as follows:

$$ASS = \frac{2(1 - p + p^2)}{3} \qquad (a)$$

$$AN = \frac{1 + 2p - 2p^2}{3} . \qquad (b)$$

The natural logarithm of the expression representing the probability of obtaining such a family expressed in terms of the expected class frequencies and the corresponding observed numbers would be:

$$\log P = L = a \log \frac{(2 - 2p + 2p^2)}{3} + b \log \frac{(1 + 2p - 2p^2)}{3}.$$

By differentiating log P with respect to p, setting this score equal to zero, and solving for p, this expression evaluates to

$$p = 1/2 - 1/2 \sqrt{\frac{3a - 3b}{a + b}}. \quad (1)$$

The amount of information per F_2 individual can be computed for this statistic by applying FISHER'S general formula for the determination of this quantity

$$i = \frac{2(1 - 2p)^2}{(1 - p + p^2)(1 + 2p - 2p^2)} \quad (2)$$

and the standard error of p is computed from the relationship

$$SE_p = \sqrt{\frac{1}{Ni}}.$$

Formulas (1) and (2) hold whether the dominant gene enters the cross from the normal parent or from the parent homozygous for the interchange. This can be demonstrated by substituting (1−p) for p in the expected frequencies a and b. The percent recombination between semisterility and a factor pair in which only two classes are available has been determined by these equations. The estimate of the linkage intensity between the lethal seedling factor pair *Xc*, *xc* and semisterility has been made on the assumption that the VI linkage group is involved in interchange 301. Additional information is necessary to verify this supposition and to increase the accuracy of the estimate of p if linkage is present.

Characterization of the Factor Pair Lg_3, lg_3

The original parent 293 was segregating for the character green vs. light green seedlings (Lg_3, lg_3) which had not been associated with any of the seven linkage groups in barley. As previously stated, the character is a recessive seedling lethal. Upon emergence the homozygous recessive seedling is light green resembling a chlorina seedling; however, this seedling does not recover. Under field conditions the seedling becomes chlorotic and dies while in about the second leaf stage. When grown under greenhouse conditions, the seedling survives to a later stage of growth but dies before maturity.

The character is considered to be controlled by a single factor pair. Evidence to support this conclusion is found in the satisfactory fit to a 3:1 ratio of 438 normal seedlings to 128 light green seedlings and in the fact that 0 percent recombination exists between semisterility and the Lg_3 locus in crosses involving

the interchanges 300 and 301. In the cross of 293×300, linkage is present between the factor pair Lg_3, lg_3 and the factor pairs V, v and K, k respectively, while in the cross 293×301 linkage is evident only between the factor pairs Lg_3, lg_3 and K, k (tables 3 and 5). The Lg_3 locus is in the IV linkage group. F_3 data will be necessary to determine the locus of this factor in the IV chromosome.

DISCUSSION

Discrepancies may exist between linkage values obtained from crosses involving an interchange and those found in the normal diploid. In most cases crossing over is reduced in the region of the break. LAMM (1948) noted in peas that the recombination between the genes *St* and *B* was reduced to 12.8 percent in an interchange heterozygote as compared to about 26 percent as determined for the diploid. He postulates that this reduction was due to the position of these genes in the interstitial segment, that is, between the centromere and the break. SUTTON (1935) has shown in peas that a chiasma in this region gives a figure-8 configuration and that distal to the chiasma, chromatid disjunction results. As shown in figure 1A, when zigzag disjunction at the centromere occurs, a chiasma between a marker gene and the break gives rise to a cross-

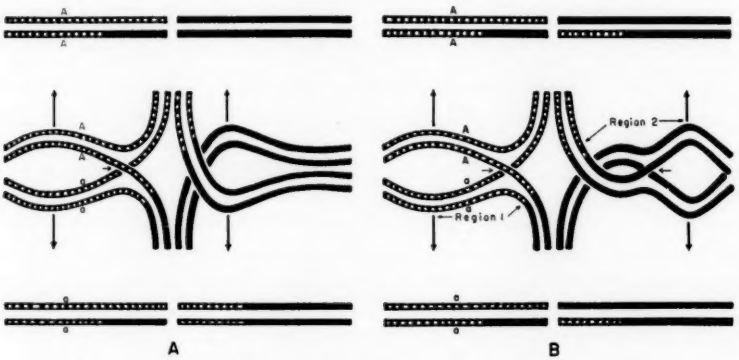


FIGURE 1.—Illustration of the effect of chiasmata in interstitial segments on recombination in the segments. For explanation see text.

over chromatid which is included in a non-functional gamete, thus reducing the amount of recombination. Considering the three possible types of centromere disjunction zigzag (A), the corresponding non-zigzag (B), and a third non-disjunctional type (C) and their corresponding frequencies a, b, and c respectively (a+b+c=1), LAMM presented the formula

$$p_T = \frac{bx}{2a - ax + bx} \tag{3}$$

to depict this condition where p_T is the recombination value and x is the chiasma (crossover) frequency in this interstitial segment. The recombination

value p in this segment (region 1) also will be influenced by the chiasma frequency z in the opposite interstitial segment (region 2). For example, it is apparent from figure 1A that if only A type disjunction occurs and if the chiasma frequency in the other interstitial region is zero, all gametes carrying a crossover between the marker gene and the break would be sterile. However, if a simultaneous crossover in region 1 and 2 occurs (figure 1B), the recombination gametes could be recovered. As the chiasma frequency in region 2 approaches unity, the recombination value obtained for region 1 would approach that found in the normal diploid since crossover and noncrossover chromatids are included in sterile gametes in equal proportion. Thus, the equation derived to include the effect of crossing over in region 2 would be

$$p_T = \frac{bx - \frac{bxz}{2} + \frac{axz}{2}}{2a - ax + bx - az + bz + axz - bxz} \tag{4}$$

When crossing over in region 2 does not occur, z is zero, and formula 4 reduces to formula 3 as presented by LAMM.

Since all gametes resulting from C type disjunction will be nonfunctional irrespective of the chiasma formation in any part of the configuration, the frequency of C type disjunction does not influence the recombination value. It does, however, influence the amount of sterility. The frequency of the gametic sterility is given by

$$S = \frac{2b - bx + ax - bz + az + bxz - axz + 2c}{2} \tag{5}$$

By substituting various values for a, b, c, x, and z in formulas 4 and 5, the effect of each of these factors on the percent recombination and on the percent sterility may be determined. These values are presented in table 7.

It appears from table 7 that as long as the frequency of A and B are equal, the percent recombination is not influenced by the chiasma frequency in either of the two interstitial regions or by the frequency of the C type of orientation and equals that found in the normal diploid ($p=x/2$) as LAMM (1948) has shown. When the frequencies of A and B orientations are unequal, both the recombination value and the percent sterility depend upon the chiasma frequencies in both regions and on the type of disjunction. As the chiasma frequency in region 2 approaches 1.00, the percent recombination in region 1 approaches that of the diploid. In order to obtain a sterility greater than 50 percent, either C orientations must be present, or the frequency of B exceeds A. Furthermore, in order to obtain a sterility of less than 50 per cent, the frequency of A type disjunction must exceed the sum of the other two.

It should perhaps be pointed out that this behavior applies only to interstitial segments, although a recombination value between a factor pair located outside of this region and a factor pair within this segment or between this factor pair and the break will be affected proportionately to the amount of interstitial region involved. For simplicity, the effect of a number of other fac-

tors (see BURNHAM, 1948a, 1948b) such as multiple chiasmata, variability in position of the center of the synaptic complex, frequency of 3-1 disjunction, possible effects of interstitial segments on the type of disjunction, and the possible effect of chiasmata interference in these two regions has been ignored.

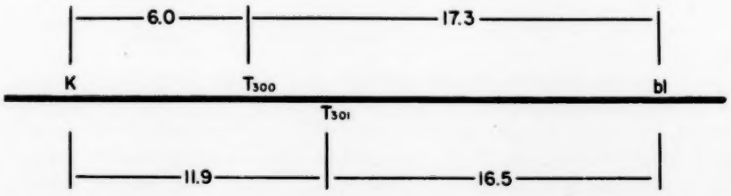
TABLE 7

The effect of frequencies of different types of disjunction and the effect of chiasma frequencies in the two interstitial regions on percent recombination in region 1 and on sterility in an interchange heterozygote.

FREQUENCY OF DISJUNCTION			CHIASMA FREQUENCY IN REGION 1 (x)	CHIASMA FREQUENCY IN REGION 2 (z)						% RECOMBINATION IN A DIPLOID WITH CHIASMA FREQUENCY AS IN REGION 1
				z=0		z=.5		z=1.00		
A	B	C		% RECOMB.	% STERILITY	% RECOMB.	% STERILITY	% RECOMB.	% STERILITY	
50	50	0	0.0	0.0	50.00	0.0	50.00	0.0	50.00	0
50	50	0	0.5	25.0	50.00	25.0	50.00	25.0	50.00	25
50	50	0	1.0	50.0	50.00	50.0	50.00	50.0	50.00	50
75	25	0	0.0	0.0	25.00	0.0	37.50	0.0	50.00	0
75	25	0	0.5	10.0	37.50	16.7	43.75	25.0	50.00	25
75	25	0	1.0	25.0	50.00	37.5	50.00	50.0	50.00	50
100	0	0	0.0	0.0	0.00	0.0	25.00	0.0	50.00	0
100	0	0	0.5	0.0	25.00	10.0	37.50	25.0	50.00	25
100	0	0	1.0	0.0	50.00	25.0	50.00	50.0	50.00	50
40	40	20	0.0	0.0	60.00	0.0	60.00	0.0	60.00	0
40	40	20	0.5	25.0	60.00	25.0	60.00	25.0	60.00	25
40	40	20	1.0	50.0	60.00	50.0	60.00	50.0	60.00	50
50	25	25	0.0	0.0	50.00	0.0	56.25	0.0	62.50	0
50	25	25	0.5	14.3	56.25	19.2	59.38	25.0	62.50	25
50	25	25	1.0	33.3	62.50	41.7	62.50	50.0	62.50	50

Nevertheless, for the purpose of considering gene position in relation to the break and the centromere and in studying sterility effects, the discussion may be of some value.

Interchange heterozygotes in barley are about 25 percent sterile, hence there must be at least 75 percent A type disjunctions resulting in unequal frequencies of the A and B types. Under these conditions a certain amount of reduction or masking of recombination values is expected in the interstitial segments. From the data in table 5, the linkage relations in group IV obtained from interchanges 300 and 301 may be summarized as follows:



It is impossible, from these data, to predict the degree of masking that may have occurred in these calculated linkage values. Unfortunately, recombination values between the factor pairs *K*, *k* and *Bl*, *bl* as determined by different investigators are inconsistent. ROBERTSON *et al.* (1947) list 44.0, 24.7, 40.6, and 22.0 as recombination values for this region. If the lower values are accepted, it is evident that these two breaks must be located between *K* and *bl*, and since no masking is apparent, the centromere of chromosome IV must be near the breaks, and asynapsis must be negligible. If the larger values are to be accepted, the position of the two breaks must still be between *K* and *bl*, but the position of the centromere can be inferred only indirectly. The reduction in recombination in the diploid (40–44 percent) to that in interchanges 300 and 301 (23–28 percent) is considerable, thus the degree of masking may be roughly estimated to be from 12 percent to 21 percent. Unless there is appreciable asynapsis, the interchange heterozygote must have a chiasma frequency of 24 percent to 42 percent which would apparently require a very high proportion of A type disjunction in order that the sterility (table 7) did not exceed 25 percent as is the case in barley. This suggested the possibility of completely zigzag orientation in barley. If this were found to be true, there would be complete masking in an interstitial segment unless some crossing over occurred in the other interstitial segment. This again would indicate that the centromere is between *K* and *bl*. The break in interchange 301 is to the right of the break in interchange 300 as illustrated, yet only a small reduction in the percent recombination between *bl* and the interchange is found as compared to the increase in percent recombination between *K* and the break position suggesting further that the centromere is on the segment carrying the *Bl*,*bl* pair.

The lethal seedling character conditioned by lg_3 is located between *K* and *bl* which places the Lg_3 locus in the region of the two light green seedlings designated by ROBERTSON, WIEBE, and SHANDS as Lg_2, lg_2 and Lg_4, lg_4. The fact that no recombination between the two interchange breaks and the Lg_3 locus was observed suggests that either the position of this factor pair is very close to the break or it is between the break and the centromere.

SUMMARY

The linkage groups I and IV are involved in the interchange 300, while linkage group IV and possibly VI are in the interchange complex 301. The linkage intensities between a factor pair and an interchange break were computed by the product method for four class F_2 data. The locus of the lethal seedling factor, green vs. light green seedling (Lg_3, lg_3), is in the IV linkage group between the factor pairs *K*, *k* and *Bl*, *bl* and is located in the region of the interchange breaks 300 and 301. F_3 data will be necessary to establish the locus of this factor.

In linkage values obtained from an individual heterozygous for an interchange, the recombination value computed for a chromosome segment which contains, or is contained within, an interstitial region is influenced by the chiasma frequencies in the two interstitial regions, the frequencies of the A

and B types of chromosome disjunctions, and the position of this segment relative to the centromere. The frequency of gametic sterility is affected by the chiasma frequencies in the two interstitial segments and the frequencies of three possible types of chromosome orientations. Formulas were derived and presented depicting these conditions. This information has been used in postulating gene position in relation to the break and the centromere and in hypothesizing the chromosome behavior in the interchange heterozygotes of barley.

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INFLUENCE OF TEMPERATURE ON THE EXPRESSION OF POSITION EFFECTS IN THE SCUTE-8 STOCK OF DROSOPHILA MELANOGASTER AND ITS RELATION TO HETEROCHROMATIZATION

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IN *Drosophila melanogaster* translocation of a euchromatic chromosome region to a heterochromatic is frequently accompanied by an effect, known as a position effect, on the activity of genes located near the point of breakage and reunion.

It has been assumed (e.g. by SCHULTZ 1941; PROKOFYEVA-BELGOVSKAYA 1945, 1947) that position effects of genes located in euchromatic regions are due to, or closely correlated with, "heterochromatization" of the bands (as observed in the salivary gland chromosomes) where the affected loci are supposed to be situated.

In the papers cited, PROKOFYEVA-BELGOVSKAYA gives data on the influence of temperature on the condition of the chromosomes in the scute-8 (sc^8) stock. The region studies was the extreme left end of the X chromosome, including bands 1A1–1B1.2 (BRIDGES' map). By virtue of the long scute-8 inversion, which includes the loci of scute (*sc*) and bobbed (*bb*), these bands while still at the left end of the chromosome, are adjacent to the heterochromatic region 20 ABC.

PROKOFYEVA-BELGOVSKAYA'S analysis of the salivary gland chromosomes of female larvae heterozygous for the scute-8 inversion, raised at different temperatures, showed that heterochromatization of the region 1A1–1B1.2 in the scute-8 X chromosome was most frequent at 25°C (71 percent of the nuclei showed the effect), while at 30°C and 14°C this region had the normal euchromatic structure in a majority of the nuclei examined (63 percent and 67 percent, respectively). The results indicate a significant effect of temperature on the phenomenon of heterochromatization.

In view of these results, it seemed of interest to study the influence of temperature on the manifestation of position effects associated with the scute-8 inversion.

The influence of temperature on some position effects is already known. Thus, in the position effect associated with In(2L)40D, affecting the eye, HINTON (1949) finds the most extreme manifestation at 17–23°C in a tested range from 15° to 29°C, with increasing normality as the extremes of temperature are approached. In T(1; 4) w^{258-18}, a translocation between the X and fourth chromosomes with a position effect at the white (*w*) locus, the eye deviates more from normality at 25°C than 29°C (DEMEREC and SLIZYNSKA 1937), and deviates still more at 18°C than at 25°C (author, unpublished). Again, unpublished data of the author show no difference in phenotypic ex-

pression of a light (*lt*) position effect, associated with T(1; 2)w^{258-39} at 17°, 25°, and 29°C. As a final example, position effects at the *cubitus interruptus* locus are more extreme at lower temperatures (STERN, MACKNIGHT and KODANI 1946), following the same pattern as the recessive mutant *cubitus interruptus* and the white-mottled 258–18 position effect.

In the first two examples given, the affected loci are located in euchromatic regions which have been transferred to heterochromatin, in the last two the affected loci normally lie near the centromere, and have been removed from a heterochromatic to a euchromatic region. Even from these few examples, it is clear that the influence of temperature on position effects is not consistent. If one assumes a direct relationship between position effect and heterochromatization (or the converse "euchromatization" of heterochromatin), it follows, either that the effect of temperature on heterochromatization varies with different chromosome regions, or that the phenotypic expression of some position effects is modified by temperature, not through its influence on heterochromatization, but through an effect on some other stage in the chain of events which link together the gene and its phenotypic effect.

This paper deals specifically with the influence of temperature on two bristle characters of the scute-8 stock—the Hairy-wing (*Hw*) effect and the scute (*sc*) effect.

The Hairy-wing, or extra bristle effect, is presumably associated with the band 1B1.2 of the X chromosome (see DEMEREC and HOOVER 1939), which is included in the region studied by PROKOFYEVA-BELGOVSKAYA, and adjacent to heterochromatin of 20 ABC.

The scute, or missing bristle effect, is probably associated with the band 1B3.4 (see BRIDGES and BREHME 1944), which is not included in the region in question, but has been transferred to the chromocenter, where it too is adjacent to heterochromatin.

MATERIAL AND METHODS

Homozygous females of the scute-8 stock were mated to *sc cv v f* males (*sc* = scute, *cv* = crossveinless (wing), *v* = vermilion (eye), *f* = forked (bristles)), and were then kept in half-pint bottles at each of the following temperatures: 14°, 18°, 25° and 30°C. The stocks used were not isogenic. The parents of flies raised at 18°, 25° and 30°C were taken from a single bottle of each of the stock cultures within a few days of each other. Female parents were thus of the same generation and likewise male parents. The flies at 18°, 25° and 30°C were raised on a single batch of medium. It was not possible to raise flies at 14°C simultaneously with those raised at the other temperatures. The 14°C cultures were raised on a different batch of medium, from parents derived from the same stock culture bottles and separated not more than three generations from those used in the other experiments.

In order to reduce sampling variations, flies from three or more bottles were counted at each temperature, thus multiplying the number of parents for each sample.

F_1 females (sc^8/*sc cv v f*) and males (sc^8) were scored separately, with regard

to four characters, viz:—1) extra thoracic bristles (*Hw* effect); 2) presence or absence of anterior and posterior scutellar bristles; 3) presence or absence of anterior and posterior supra-alar bristles; 4) presence or absence of anterior, median and posterior sternopleural bristles. These particular bristles were chosen because they are supposed to be the most frequently affected in the scute-8 stock (BRIDGES and BREHME 1944).

Two hundred females and two hundred males were scored at each temperature for all the above characters, with the following exceptions: At 25°C, only 75 of the 200 females and 75 of the 200 males, chosen at random, were scored for scutellar bristles. At 30°C, only 75 of the 200 females were scored for scutellar bristles; while the total number of males examined was 80, and only 65 of these were scored for scutellar bristles.

RESULTS

The data obtained are presented in table 1 and in the form of histograms in figure 1. The number of flies showing a given abnormality is shown in the histograms as a percentage.

TABLE 1

Numbers and percentages of individuals with extra bristles, or lacking specific bristles, at different temperatures.

TEMPERATURE °C	EXTRA BRISTLES				ANT. SCUTELLAR				POST. SCUTELLAR			
	♀		♂		♀		♂		♀		♂	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
14	41	20.5	174	87	0	0	0	0	1	1.3	1	1.5
18	52	26	178	89	0	0	0	0	0	0	0	0
25	9	4.5	157	78.5	1	1.3	6	8	2	2.7	5	6.7
30	68	34	77	96	51	68	64	98.4	7	9.3	54	84.6

	ANT. SUPRA-ALAR				POST. SUPRA-ALAR			
	♀		♂		♀		♂	
	NO.	%	NO.	%	NO.	%	NO.	%
14	5	2.5	100	50	0	0	8	4
18	0	0	48	24	1	0.5	2	1
25	0	0	12	6	1	0.5	2	1
30	0	0	33	41	0	0	0	0

Owing to the much lower incidence of abnormalities in females than in males, it has proved necessary to deal largely with the data for males, but, as will be seen, the data for females provide corroborative evidence in certain important instances.

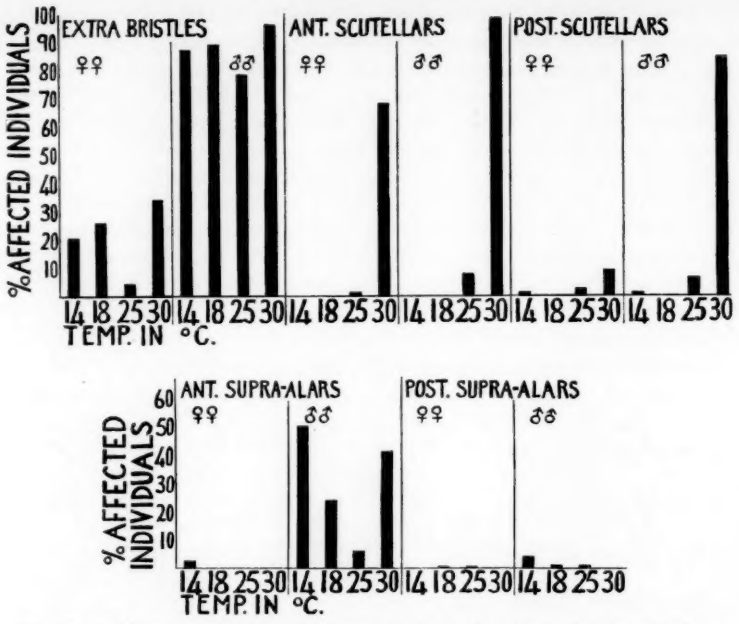

FIGURE 1.—Histograms showing percentages of individuals with extra bristles, or lacking specific bristles, at different temperatures.

The results may be summarized as follows:

Extra bristles. Females and males show similar reactions to different temperatures, apart from the lower frequency of individual females with one or more extra bristles. The data for females show a significant temperature effect as between 14° C and 25° C, and as between 25° C and 30° C $\left(\frac{\text{Diff.}}{\text{S.E.}} = 4.8 \text{ and } 7.3\right.$ respectively, $P = 10^{-5}$ and 10^{-8} respectively). The males show a possibly significant difference between the proportions of affected individuals at 14°C and 25°C $\left(\frac{\text{Diff.}}{\text{S.E.}} = 2.29,\ P = 0.02\text{–}0.03\right)$ and a clear cut difference as between 25°C and 30°C $\left(\frac{\text{Diff.}}{\text{S.E.}} = 3.6,\ P = 10^{-3}\right)$.

Scutellar bristles. All scutellar bristles appear to follow the same general pattern in relation to temperature in both females and males, with a maximum of missing bristles at 30°C, fewer at 25°C and virtually none at the lower temperatures.

The differences in anterior scutellars between 25°C and 30°C are significant

for both females $\left(\frac{\text{Diff.}}{\text{S.E.}} = 11.1, P = 10^{-9}\right)$ and males $\left(\frac{\text{Diff.}}{\text{S.E.}} = 10.4, P = 10^{-9}\right)$.

For posterior scutellars, the difference between 25°C and 30°C is not significant for females $\left(\frac{\text{Diff.}}{\text{S.E.}} = 1.74,\ P = 0.08\text{–}0.09\right)$, but significant for males $\left(\frac{\text{Diff.}}{\text{S.E.}} = 9.27,\ P = 10^{-9}\right)$.

Supra-alars. A very small number of females with missing anterior supra-alars was obtained but these were all raised at 14°C, the temperature at which the greatest number of affected males was found. The males show a minimum of affected individuals at 25°C, with greater effects at the temperature extremes. The differences between 14°C and 25°C and between 25°C and 30°C are highly significant $\left(\frac{\text{Diff.}}{\text{S.E.}} = 9.75 \text{ and } 7.1 \text{ respectively. P in both cases} = 10^{-9}\right)$.

Missing posterior post-alars were infrequent and the numbers are too small to justify any conclusion about the influence of temperature.

Sternopleurals. Absence of any of the sternopleurals occurred very rarely (in one male at 18°C and in four females and one male at 14°C) and is not represented in the table and histograms.

DISCUSSION

While it would have been desirable to have used isogenic stocks in these experiments, it seems probable that the conclusions drawn below are valid. As it was pointed out under Materials and Methods, the experiments at 18°, 25° and 30°C were carried out under otherwise identical conditions. The comparisons between flies raised at 25° and 30°C are therefore legitimate. Although this is not true of comparisons between 14° and 25°C, these are put on a sounder basis by the fact that, wherever they show a significant difference there is also a significant difference in the same direction between flies raised at 18° and 25°C.

It is clear from what was said in the introduction that one might expect to find correlation between PROKOFYEVA-BELGOVSKAYA'S data on heterochromatization and the phenotypic expression of the extra bristle (Hairy-wing) effect, located in the region 1A1–1B1.2 which she studied.

PROKOFYEVA-BELGOVSKAYA found a maximum of heterochromatization at 25°C. One would assume, therefore, that there should be a maximum abnormality of phenotypic expression at this temperature for genes located in the 1A1–1B1.2 region. The present data show that the extra bristle effect has an opposite relation to temperature, with minimum abnormality at 25°C and maxima at the more extreme temperatures.

The scute locus does not lie within the region studied by PROKOFYEVA-

BELGOVSKAYA and would not necessarily be expected to show a correlation with her data.

The different bristles affected do not all show the same relation to temperature. For all except the scutellar bristles the incidence of abnormalities in the females was negligible, and it might be argued that there is some doubt as to whether all missing bristles in the scute-8 males are attributable to an effect of the scute locus, or whether other bristle-determining loci in the same region may not be affected. Similar results were found by CHILD (1935), however, in a temperature study of the scute-1 stock, a mutant scute stock in which the X chromosome is normal and no position effects are involved; so that it seems justifiable to consider the scute locus responsible for the whole missing bristle complex.

In this case, it is evident that one cannot expect any simple relationship between the phenotypic manifestation of the scute effect and heterochromatization of the scute end of the inversion in the scute-8 stock.

The effect of temperature on the phenotype of flies carrying the Hairy-wing duplication is not known. The only bristles affected by the scute locus which are considered in common in this study and CHILD's (*loc. cit.*) are the scutellars, and the data for the anterior scutellars in scute-1 and scute-8 differ markedly. We are, therefore, not in a position to judge the nature of the temperature effect on these position effects—whether it influences them *qua* position effects, or merely affects one or more steps in the chain of "bristle-forming reactions" initiated by the pertinent loci.

SUMMARY

The effect of temperature on the expression of the Hairy-wing and scute position effects in the scute-8 stock was studied in males and females from the cross $sc^8/sc^8 \times sc\ cv\ v\ f$, raised at 14°, 18°, 25° and 30°C.

The Hairy-wing effect occurred with least frequency at 25°C and greater frequency at the more extreme temperatures on either side of 25°C.

The different bristles affected by the scute locus were not consistent in their relationship to temperature, scutellar bristles being most frequently absent at the higher temperatures and anterior supra-alars at the lower.

It is concluded that the expression of the Hairy-wing position effect is not directly related to heterochromatization, and that there can be no simple relationship between the manifestation of the scute effect and heterochromatization.

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ANALYSIS OF PLEIOTROPISM AT THE W-LOCUS IN THE MOUSE: RELATIONSHIP BETWEEN THE EFFECTS OF W AND W^v SUBSTITUTION ON HAIR PIGMENTATION AND ON ERYTHROCYTES[1]

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THE dominant-spotting genes of the mouse, *W*, W^v, and *w*, have been studied extensively by DEABERLE (1925, 1927), GOWEN and GAY (1932), LITTLE and CLOUDMAN (1937), GRÜNEBERG (1939, 1942a), and FEKETE, LITTLE and CLOUDMAN (1941). They are especially noteworthy in that the six possible genotypes differ in a regular manner in a number of apparently independent characteristics: size and number of erythrocytes, development and functioning of gonads, and intensity and location of hair pigment (table 1), as well as in such probably secondary characteristics as viability. Attempts to trace these separate effects back to a common embryological origin should give

TABLE 1

General description of genic effects at the W, W^v, w locus in the mouse.

GENOTYPE	ERYTHROCYTES		VIABILITY	GONAD DEVELOPMENT	PIGMENT	
	NUMBER	SIZE			INTENSITY	SPOTTING
ww	normal	normal	normal	normal	full	none
Ww	normal	normal	normal	normal	full	belly spot
WW	extreme reduction	macrocytic	90% die 0–7 days without postnatal development	deficient	white	?
W^vw	slight reduction	slightly macrocytic	normal	normal	diluted	belly spot
W^vW^v	considerable reduction	macrocytic	normal	deficient	white	?
WW^v	greater reduction	macrocytic	53% die 0–21 days, others fairly normal	deficient	white	?

valuable information on the paths of gene action, nature of causes of congenital macrocytic anemia, and nature of genic effects on gonad development and pigment intensity and location. Analysis is greatly aided by the existence of two dominant deleterious alleles, *W* and W^v, which make it possible to compare the effects of six rather than the usual three levels of gene "dosage."

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Even before embryological studies are undertaken, evidence as to the closeness of relation between two end-effects of the genes can be garnered from comparisons of the order and magnitude of effect of the six "dosage" levels upon the end-product in question. It is such evidence for pigment intensity and erythrocyte count which is presented in this paper. The blood picture will be presented more fully elsewhere, and only those counts and measurements pertinent to these studies included here. However, this is intended as a full presentation of all data on intensity of pigmentation available purely from observation of completed hairs.

The concept of pleiotropism, or multiple effects of single genic substitutions, is, of course, of great interest to the student of gene action. One aspect of the problem has been described recently by GRÜNEBERG (1938a, b, 1943a): the distinction between "genuine" pleiotropism, where the gene involved has more than one primary effect ("doing two things directly by means of different mechanisms"), and "spurious" pleiotropism, where there is "unity of gene action," the gene having a single primary action with two effects traceable to a common cause or with one effect subordinated to the other. He has presented these possibilities clearly in diagram form (1943a). As he correctly points out, "the existence of genuine pleiotropism has never been conclusively demonstrated, while the existence of spurious pleiotropism is beyond doubt." He himself has demonstrated clearly by extremely able analysis (1938b, 1943a) the great contribution studies of "pedigree of causes" can make to our knowledge of *paths* of gene action.

For the purpose of this paper, however, the question of distinction between "genuine" and "spurious" pleiotropism seems less pertinent than certain other considerations, and it seems advisable to present another classification of types of pleiotropism (figure 1). Of these possibilities, A2 and D correspond exactly with GRÜNEBERG'S classification of "genuine" pleiotropism. In all other classes there is a single primary gene action in the strictest sense that only one original gene product need be assumed to enter and act in the cytoplasm. The chief difference between the present chart and GRÜNEBERG'S earlier one (1943a) is the introduction of the possibility that "unity of gene action" could exist without "tissue or cell specificity." It is logically possible, as listed in C of figure 1, that the same original gene product could by its intrinsic nature be active in two types of cells, long separated in development and different in structure. The gene in question might either act on the same substrate which has been independently produced in two different localities, or, perhaps more probably, it might be general enough in its action to affect two substrates. Certain oxidative enzymes might be examples of the second possibility. Where two types as different in origin and function as erythrocytes and matrix cells of the hair-follicle are involved it seems this category of pleiotropism must be considered. In one sense there is unity of gene action, since the gene releases only one product. In another sense there is real pleiotropism, as this one gene product is "doing two things directly by means of different mechanisms."

Brief examination of table 1 shows that there is parallelism in the effect of genes of the *W*-series on erythrocytes and on pigmentation to the extent that

the three most anemic genotypes (WW, W^vW^v, and WW^v) are all completely lacking in pigment in the hair, while the three types with higher blood counts are all pigmented. It is futile, however, to study these three white extreme anemic genotypes further for correlation between the two characters, as there is no variability in pigmentation. The three pigmented genotypes are more promising. The normal homozygote, *ww*, has of course, full color intensity and

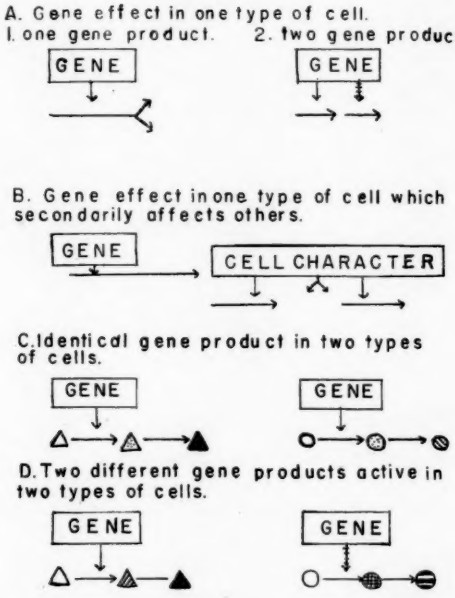
A. Gene effect in one type of cell.
1. one gene product.
2. two gene products
GENE
GENE
B. Gene effect in one type of cell which secondarily affects others.
GENE
CELL CHARACTER
C. Identical gene product in two types of cells.
GENE
GENE
D. Two different gene products active in two types of cells.
GENE
GENE

FIGURE 1.—Theoretically possible types of pleiotropism

normal blood picture. GRÜNEBERG, however, after having first described both heterozygotes as normal in blood picture (1939), changed his view and later (1942a) concluded that although the blood picture was completely normal in *ww* and *Ww*, the W^vw genotype had a lower number of erythrocytes, and a larger mean cell volume, indicating that W^v was partially dominant in its effect on blood, while W was completely recessive. *A priori* this seems improbable, since the effect of two doses of WW is more extreme than that of two W^vW^vs. However, preliminary experiments at that time (1943) by the author on heterogeneous material showed that in each of the five stocks tested, in litters segregating for W^vw and *ww* there were significantly fewer erythrocytes in the W^vw littermates, as previously indicated by GRÜNEBERG. As shown in table 1, these three pigmented genotypes also are distinguishable by their pigmentation. Both types of heterozygote always differ from the normal homozygote by the presence of a white belly spot. However, black or full-

color agouti animals of the *Ww* and *ww* genotypes are indistinguishable from the dorsal surface, and the pigment on the colored portion of the ventral surface is usually though not universally of full intensity in the *Ww* heterozygote. Black $W^v w$ heterozygotes show quite marked reduction in dorsal pigmentation, and invariably even greater reduction on the pigmented portion of the belly. Thus W^v appears partially dominant in a second type of effect where *W* is completely recessive. This apparent parallelism in dominance relations of the two alleles on pigment intensity and erythrocyte number suggested that careful quantitative evaluation of both characters might be helpful in analyzing the nature of the pleiotropism. These stimulating possibilities have led to two types of experiments. First, breeding experiments were undertaken to place both dominant genes on the same uniform genetic background so that differences observed with genic substitutions could definitely be attributed to those genes and not to modifiers which might differ between stocks. It was felt that this step was particularly essential for the comparisons of erythrocyte number and size. Second, in order to obtain a more thorough understanding of the W^v effect on pigmentation intensity, crosses were made leading to litters segregating for $W^v w$ and *ww* on five different colored backgrounds, involving three qualitatively different pigments (black-fuscous, brown, and yellow), and two other types of pigment reduction (pink-eyed pale sepia, C-series) (RUSSELL 1946). It was hoped that with the existing knowledge of the physiology of pigmentation, histological studies of these particular stocks would test the type of pigment reduction involved, and that accurate counts and measurements of the pigment granules in segregating littermates in each stock would lead to further conclusions as to the basic nature of the gene actions involved.

In the first type of experiment mentioned above, the two dominants *W* and W^v were introduced into the Jax C57 black strain. The animals used in the erythrocyte counts recorded here were from the second and third back-cross generations, so within each littermate comparison, 75 to 88 percent of the genes other than *W*-alleles should be identical. The exact nature of the residual heterozygosity may differ from litter to litter. Determinations are recorded on seven litters segregating for $W^v w$-*ww* (table 2) and on eight segregating for *Ww*-*ww* (table 3). Two erythrocyte counts (number per cu mm) and two haematocrit readings (proportion of cell volume to total blood volume) were made for each animal, using blood obtained from the tail (heated) of non-anaesthetized individuals between four and five weeks of age. The erythrocyte counts were made with a Levy haemacytometer and regulation red-counting pipettes, and the accuracy of sampling can be judged from the mean difference between the two samples taken from each animal, 0.43×10^6 for the 50 animals involved in this experiment. The haematocrit readings were made by diluting the blood with 1.3 percent sodium oxalate in Van Allen haematocrit tubes, and centrifuging in a clinical centrifuge at 2,700 rpm for one-half hour. As pointed out by PONDER (1944) the values obtained for proportional blood volume vary somewhat with the conditions of centrifugation and dilution oxalate in particular differing from other diluents. Thus the mean cell volumes obtained in this study may not be strictly comparable to those obtained by

other workers (GRÜNEBERG 1939, 1942a), but they should give satisfactory comparison within the current experiment. The personal accuracy may be judged from a mean difference of 1.2 percent between the two samples taken from each of the 50 animals used here. In as far as possible, two animals of each genotype were taken from each litter, and the values given in tables 2 and 3 are the means of the two samples from each individual.

Using means of litters as a basis, paired comparisons were made for each type of measurement of $W^v w$ and ww segregants. In each, there was a signifi-

TABLE 2

Erythrocyte counts, haematocrit readings, and determinations of mean cell volume of 4–5 week old animals in litters segregating for $W^v w$ and ww.

LITTER	rbc×10⁶	ww haem. %	M.C.V., mu³	rbc×10⁶	$W^v w$ haem. %	M.C.V., mu³
1	8.55	40.8	47.7	7.32	43.3	59.2
1	8.72	41.3	47.4	6.48	39.0	60.2
2	10.68	44.8	42.0	7.87	39.0	49.6
2		47.0	44.0	9.08	42.5	46.8
3	9.26	41.5	44.8	6.41	38.8	60.5
3	7.89	39.8	50.4	8.85	36.5	41.2
4	9.39	36.0	38.3	6.95	31.5	45.3
4	8.87	36.5	41.1	8.08	—	—
5	9.30	45.3	48.7	7.68	43.8	57.0
6	7.63	40.5	53.1	7.05	36.0	51.7
7	7.93	38.5	48.5	6.67	37.0	55.5
7	7.24	40.0	55.2	6.72	41.0	61.0
mean	8.72	41.0	46.8	7.43	38.9	53.5

cant difference between the genotypes (erythrocyte counts, ww higher than $W^v w$, haematocrit reading, ww higher than $W^v w$, mean cell volume, $W^v w$ (macrocytic) higher than ww, probability of chance deviation less than 0.01 in each case). The same three types of paired comparisons were made of Ww and ww segregating littermates, but in these no significant differences were found (probability of chance difference in erythrocyte number, 0.5, of chance difference in haematocrit percentage, 0.3, of chance difference in mean cell volume, 0.3). Thus the observation that W^v is partially dominant in its effect on blood has been confirmed, using material well on its way toward homogeneity. With similar material, W has been shown to have no dominance. One fact which has been added by this study is that in this material, at least, in spite of the definite macrocytosis of $W^v w$ heterozygotes, there is still sufficient reduction in cell number to result in a significant decrease in total cell volume.

Thus the first type of experiment, although still not completely finished, has demonstrated rather clearly the difference in dominance relations of the two alleles in their effect on the flowing blood. Further studies on more isogenic material will be needed to measure the exact degree of W^v dominance.

Investigations on the second problem, the nature of the effects of W^v substitution upon a variety of genetically different color types, and the question of existence of an effect of W substitution in pigment intensity, have been carried out, using five different color backgrounds for the W^v substitutions, only one for W substitution. No attempt was made here to develop isogenic stocks, as in general the major color genes are extremely regular in their manifestation.

Samples of hair were taken from the rump of eight four to five week old animals of each of the following genotypes: *aaBBCCDDPPW^{v}w; aaBBc^{ch}c^{ch}DDPPW^{v}w; aabbCCDDPPW^{v}w; aaBBCCDDppW^{v}w; A^{v}aBBCCDDPPW^{v}w-A^{v}aBBCCDDPPww;* and *aaBBCCDDPPWw-aaBBCCDDPPww.*

TABLE 3

Erythrocyte counts, haematocrit readings, and determinations of mean cell volume of 4-5 week-old animals segregating for Ww and ww.

LITTER	rbc×10^6	*ww* Haem. %	M.C.V., mu^3	rbc×10^6	*Ww* haem. %	M.C.V. mu^3
1	9.93	41.0	41.3	7.89	40.3	51.0
2	8.67	40.3	46.5	8.84	47.5	48.3
2	9.75	43.3	46.4	10.39	44.5	42.9
3	8.21	38.0	46.3	7.74	41.5	53.6
4	7.76	46.0	59.3	10.36	44.8	43.2
4	8.15	40.5	49.9	9.58	40.5	42.3
5	7.85	35.5	45.3	8.30	39.0	47.0
5	7.95	42.0	52.8	8.65	38.8	44.8
6	8.05	40.8	50.6	10.64	51.0	47.9
6	7.85	37.0	47.2	8.75	42.0	48.0
7	8.08	41.0	50.6	8.47	39.0	46.1
7	9.55	49.3	52.1	8.86	46.5	54.1
8	7.50	40.3	53.7	8.02	37.8	47.1
mean	8.41	41.2	49.2	9.04	42.6	47.4

In the first four genotypes, the differences from the corresponding *ww* genotypes were so great that it was felt no error in interpretation would result from comparing the present values with the means of corresponding *ww* types taken from unrelated animals described in the general paper of RUSSELL (1946). In the case of the last four genotypes, bracketed together in pairs, the differences between *W^{v}w* and *ww* pairs of genotypes were not obvious, and for that reason samples were taken from litters segregating for these genes to reduce extraneous variability. Cross sections and whole mounts were made of the hair samples (full grown hairs of the first pelage) by the standard methods of RUSSELL (1946). Counts of granules in a unit volume of cortex and in a single medullary cell were made at a series of levels from tip to base of the hair (4th, 10th, 20th, 30th, 40th, and 50th microscopic fields, at 1800×). The mean values at each level for each genotype are given in table 4, combined, for the first four geno-

types, with the earlier published means of corresponding *ww* genotypes. In figure 2 histograms are given comparing the mean values for $W^{v}w$ and *ww* genotypic pairs by superimposing them on the same diagram. The values for *ww* are connected by a solid line, those for $W^{v}w$ by a broken line. It will be noticed that the medullary numbers in particular are always lower in the $W^{v}w$

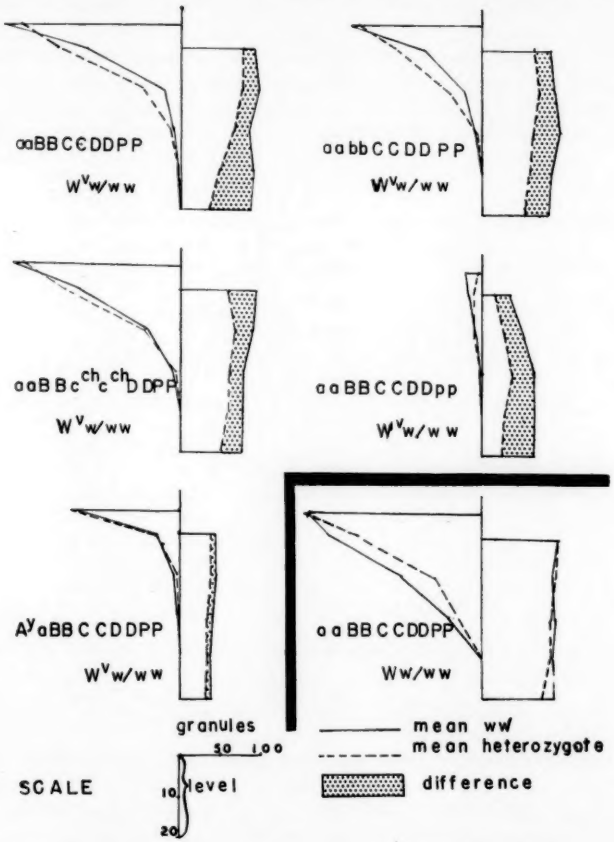

FIGURE 2.—Comparisons of numbers of pigment granules in the medulla and cortex in pairs of genotypes segregating for $W^{v}w/ww$ or Ww/ww. Each histogram compares number of granules at various levels in pairs of genotypes differing by the indicated substitution. Values to the left of vertical axis are for cortical number per unit volume, to the right, mean number of granules per medullary cell.

member of a pair, and the shaded area in each histogram represents the amount of reduction in medullary granule number with $W^{v}w$-*ww* substitution in that genetic background. It is clear that the degree of reduction, as suggested above is less in the yellow stock than in the first four $W^{v}w$-*ww* stocks. The sixth histo-

gram in figure 2, and the last column in table 4, show a littermate comparison of *aaBBCCDDPPWw-aaBBCCDDPPww*. Here no area has been shaded since casual inspection fails to show any reduction of pigment granule number. The slight degree of $W^v w$ reduction in the yellow background, and the apparent lack of reduction with *Ww-ww* substitution on a black background raise once more the question of the nature of these pigment effects. If the yellow type is significantly affected, the $W^v w$-*ww* pigment reduction is probably a general

TABLE 4

Number of pigment granules at successive levels in corresponding $W^v w$-ww and Ww-ww genotypes (cort. = granules per unit cortical volume; med. = granules per medullary cells; M = mean, E = standard error; field = width field at 1800×, approximately 85μ).

GENOTYPE	REGION	4TH		10TH		20TH		30TH		40TH		50TH	
		M	E	M	E	M	E	M	E	M	E	M	E
aaBBCCDDPPW^{v}w	cort.	195	± 4	153	±27	49	±16	5	±3	0		0	
	med.	—		81	± 5	77	± 4	65	±7	47	±7	38	±8
	cort.	219	± 7	113	±12	21	± 5	4	±3	2	±2	0	
	med.	—		93	±14	95	±11	86	±2	90	±6	87	±2
aaBBc^{ch}c^{ch}DDPPW^{v}w	cort.	189	±13	143	±19	41	±11	7	±4	0		0	
	med.	—		61	± 4	71	± 2	63	±3	67	±4	58	±4
aaBBc^{ch}c^{ch}DDPPqw	cort.	203	± 8	128	± 9	35	± 4	8	±3	0		0	
	med.	—		98	± 2	93	± 3	85	±3	80	±3	77	±5
aabbCCDDPPW^{v}w	cort.	149	± 6	112	± 9	40	±11	2	±1	0		0	
	med.	—		68	± 2	73	± 3	66	±3	62	±3	56	±4
	cort.	160	± 8	73	± 3	21	±10	4	±3	0		0	
	med.	—		82	± 3	90	± 4	95	±4	85	±4	82	±8
aaBBCCDDppW^{v}w	cort.	3	± 2	9	± 3	8	± 3	0		0		0	
	med.	—		14	± 3	30	± 4	34	±4	30	±6	27	±6
aaBBCCDDppww	cort.	18	± 3	15	± 1	4	± 2	0		0		0	
	med.	—		32	± 2	51	± 3	62	±4	71	±5	69	±5
A^{y}aBBCCDDPPW^{v}w	cort.	131	± 6	34	±12	2	± 2	1	±1	0		0	
(littermates)	med.	—		40	± 3	40	± 2	36	±3	41	±3	36	±3
A^{y}aBBCCDDPPww	cort.	111	±15	30	± 9	5	± 4	0		0		0	
	med.	—		47	± 4	46	± 3	44	±3	43	±3	44	±3
aaBBCCDDPPWw	cort.	220	±10	152	±10	60	±11	28	±6	0		0	
(littermates)	med.	—		98	± 2	94	± 2	84	±4	86	±5	75	±6
aaBBCCDDPPww	cort.	212	±10	186	±13	100	±16	35	±4	0		0	
	med.	—		97	± 2	90	± 2	94	±2	85	±3	92	±2

one, since all of the qualitatively different pigments here investigated are affected. If yellow is not significantly affected, the *W^{v}w-ww* reduction is limited to eumelanotic pigments. On the other hand, while there is no apparent regular reduction in granule number with *Ww-ww* substitution on a black background, there are points where such reduction can be found (30th and 50th field medullary granule counts) and it would be valuable to have some method of determining whether or not the over-all picture indicates significant reduction. However, statistical treatment of the data is complicated by the fact that the individual measurements which can be paired between littermates (number of granules per medullary cell at the 10th microscopic field of *W^{v}w-ww* segregants, for example) are not independent of each other, as the successive levels come from the same hair, and there may be associations with factors affecting that particular hair, or with hair type (zig-zag or awl), with particular levels of the hair, and with factors common to littermates, as well as the factors whose

TABLE 5

Analysis of variance in medullary granule number in litters segregating for A^{v}aBBCCDDPPW^{v}w-A^{v}aBBCCDDPPww.

SOURCE OF VARIATION	SUM OF SQUARES	DEGREES FREEDOM	MEAN SQUARE	F RATIO
genotype	3176	1	3176	19.05**
litters	2175	4	544	3.26*
error (a)	2334	14	167	—
levels	431	4	108	1.11
hairtypes	581	1	581	5.98*
level×hairtype	2969	4	742	7.63**
level×litter	2528	16	158	1.63
litter×hairtype	222	4	55	0.57
genotype×level	224	4	56	0.58
genotype×hairtype	3	1	3	0.02
error (b)	14202	146	97	—

* Significance at the 5% level.
** Indicates significance at the 1% level.

effects are being determined, those associated with the *W^{v}w-ww* genotypic difference. At the suggestion of EARL L. GREEN, and with a great deal of help from him which the author wishes to acknowledge here, a method for determining the contribution of each of such a complex of factors to the total variance of medullary granule numbers was developed. Contributing factors analyzed were the effects of difference in genotype (*W^{v}w-ww* or *Ww-ww*); of single factors varying among litters, levels of hair and hairtypes; and of interacting factors common to hairtype and level of hair, to litter and level of hair, to hairtype and litter, to genotype and hairtype, and to genotype and level. This method was applied only to the yellow *W^{v}w-ww* segregants, since it is only here that the reality of *W^{v}w* reduction is in doubt, and also, of course, to

the black *aaWw-ww* segregants. Results of these analyses of variance are given in tables 5 and 6. It is immediately clear from table 5 that the difference between $W^v w$ and *ww* genotypes is the major source of variance in the $W^v w$-*ww* yellow stock. The difference between the two genotypes is highly significant (F is 19.05, much greater than $F_{0.01}$, 8.86). By the same test, the difference shown in table 6 between *aaWw* and *aaww* segregants is clearly not significant. (Before leaving these tables one further significant source of variation should be mentioned, that due to factors common to level and hairtype, which is also

TABLE 6

Analysis of variance in medullary granule number in litters segregating for aaBBCCDDPPWw-aaBBCCDDPPww.

SOURCE OF VARIATION	SUM OF SQUARES	DEGREES FREEDOM	MEAN SQUARE	F RATIO
genotype	604	1	604	2.62
litters	2967	3	989	4.3*
error (a)	2535	11	230	—
levels	3827	4	957	1.66
hairtype	926	1	926	1.60
level×hairtype	412	4	103	.18
level×litters	2676	12	223	.39
litters×hairtype	1601	3	534	.93
genotype×level	1506	4	264	.46
genotype×hairtype	843	1	843	1.46
error (b)	66401	115	577	—

* Significance at the 5% level.
** Indicates significance at the 1% level.

significant at the 1 percent level ($F = 7.63 > F_{0.01} = 3.44$.) This means that in these particular stocks the degree of difference between zig-zag and awl hairs depends upon the level of the hair.) These analyses of variance have confirmed statistically the suspected difference in dominance relations between the W^v and W alleles in their effect on pigment intensity, and have indicated that the W^v dominant effect is a general one, since in the doubtfully affected yellow stock, the differences between genotypes is by far the greatest source of variation.

The fact that granule number is affected by $W^v w$-*ww* substitution in three qualitatively different pigments (black-fuscous, brown, and yellow), and in differently arranged shred-granuled *pp* types has already shown quite a bit about the nature of the $W^v w$ effect on pigment intensity, i.e., has shown its generality. Another deduction which might be drawn from a histological examination might come from a study of the effects of this gene substitution on granule size. In an earlier study of the interrelationships among variable granule attributes (RUSSELL 1949a) one of the four key pigmentation characteristics described was the degree of pigmentation, which referred to a "group

phenomenon in which a number of attributes vary together in response to one basic factor, the general degree of pigmentation." "Among the attributes conditioned by this key factor are medullary number itself . . . and size of black-fuscous granules. . . . These attributes vary with changes in general degree of pigmentation whether such changes are produced by genic substitution or by non-genetic effects such as position along the hair axis." Yellow granule size can be affected by changes in degree of pigmentation only in very extreme cases, and pink-eyed granules and brown granules have not been shown to be affected in any case as yet. Therefore, if the *W*^{v}*w-ww* effect on pigment intensity is to be classified into one of the key pigmentation characteristics, its effect on the mean size of various types of granules will help determine whether or not it is a change in the general degree of pigmentation. Table 7 compares the mean greater diameter of corresponding *W*^{v}*w*, *Ww* and *ww* types, based on littermate comparisons, and shows quite clearly that in this case *Ww-ww* substitution does not reduce the size of black-fuscous granules, while *W*^{v}*w-ww*

TABLE 7

The effect of W^{v}*w-ww and Ww-ww substitution on granule size in various genetic backgrounds. Value given is the mean and standard error of the greater diameter of 100 granules of each genotype. An asterisk indicates a significant difference.*

BACKGROUND GENOTYPE	*W*^{v}*w*		*Ww*		*ww*		*ww-W*^{v}*w*	*ww-Ww*
	M	E	M	E	M	E	diff. E	diff. E
aaBBCCDDPP	1.43	± .008	1.53	± .011	1.50	± .008	0.07 ± .012*	−.03 ± .014
aaBB$c^{ch}c^{ch}$*DDPP*	0.99	± .008	—		1.03	± .010	0.04 ± .013*	—
aabbCCDDPP	0.78	± .007	—		0.77	± .007	−0.01 ± .011	—
aaBBCCDDpp	0.55	± .031	—		0.58	± .015	−0.03 ± .020	—
A^{v}*aBBCCDD*	0.77	± .010	—		0.78	± .009	−0.01 ± .013	—

substitution significantly reduces the mean greater diameter of two black-fuscous genotypes (*aaBBCCDDPPW*^{v}*w-ww*; *aaBB*$c^{ch}c^{ch}$*DDPW*^{v}*w-ww*) and does not significantly affect brown, pink-eyed black, or yellow. This shows clearly that the effect of *W*^{v}*w-ww* substitution is very similar to other general changes in degree of pigmentation, since the effect on granule size is limited to the same types of granules.

DISCUSSION

An extensive parallelism of effect of *W*^{v} and *W* substitutions on two very different localities, (a) blood and (b) pigment-producing cells of the hair follicle, has been demonstrated. In both tissues, the double dominant types are severely affected; they, *WW*, *WW*^{v}, and *W*^{v}*W*^{v}, are black-eyed whites; they also have rather extreme macrocytic anemia, though its exact degree varies with the particular alleles present, those with *W* being the most anemic. One dose of *W*, however, has no effect either on erythrocyte count or hair pigment intensity. One dose of *W*^{v} has a slight but significant effect on both

tissues. The nature of the effect of $W^v w$-ww substitution on hair pigment intensity has been investigated sufficiently to say that it is a general reduction, affecting all the types of pigment which were studied. The basic question of interest in a study of these pleiotropic effects is the place and time of original gene action. Certainly the two types of tissues studied here, affected so similarly by the same series of genes, come from widely separated cell lineages. All mammalian haematopoietic tissues come directly or indirectly from the primitive mesenchyme (BLOOM 1938), and the main sites of haematopoiesis are successively the yolk sac, the liver, and the bone marrow. All hair pigment in the mouse is believed to come from melanophores which migrate out from the neural crest between the eighth and twelfth days of gestation (RAWLES 1947). It hardly seems possible that the gene action responsible for these widely separated effects can have taken place in a common ancestor of the mesenchyme and neural crest cells, since so many other descendants of the same cells appear to be unaffected. Rather, one effect must be dependent upon the other for its appearance (figure 1, B), or both must result from gene action independently in two types of cells (figure 1, C or D), or both tissues must be in an especially susceptible state to be affected by some gene action external to both at a particular embryonic stage (special double case of figure 1, B). The type of data given here cannot distinguish definitely among these possibilities. One possibility, of course, is that the anemia arises early enough in embryonic development to inhibit the melanophores so either their number or pigment-producing capacity is limited. (It should be noted that the extent of final *migration* of melanophores is restricted by one dose of either W or W^v, since both types always carry a belly spot. This effect is not parallel with the blood effect, however.) The concept of inhibition of development by an anemia has been used to explain the secondary effect of *ff* siderocyte anemia on the tail and on extent of pigmentation (GRÜNEBERG 1942b). In considering this possibility, however, it should be mentioned that in most cases where arrest of development at a particular time has been postulated as a manner of gene action, a skeletal effect has been involved, since some part of the axial skeleton is actively growing at almost any stage in embryonic development. The W series appears to have no effect at all on the skeleton. The third possibility, that some change external to both tissues acts similarly on the two at a critical time in development, should be considered in the same light: since no skeletal effect is involved, it is doubtful if such an outside change could be a general arrest in development; this does not exclude some other type of external change such as change in concentration of specific chemicals or alteration of the percent survival or rate of motion of wandering cells. The first step in investigating these possibilities is, of course, a determination of the time of first appearance of the anemia, and this is being carried out at present. A second possibility is that the anemia, which continues throughout life, may in some way diminish the supply to the hair follicle of some substance essential for pigment formation. (It should be remembered, however, that the actual haemoglobin level was thought by GRÜNEBERG to be the same in $W^v w$ and ww individuals due to the macrocytosis counteracting the reduction in numbers;

our data do indicate a slight but significant decrease in total cell volume.) Data somewhat pertinent to the first point and extremely important to the idea of an effect from a continuing anemia would be obtained by transferring skin between types differing in their blood pictures, as autonomy or nonautonomy of pigment deposition in transplants would determine their dependence upon the nature of the blood supply. It should also be mentioned that if such transplants should prove to be nonautonomous, it would be a definite exception to the general rule, which is that pigmentation is strictly locally determined. The only previous exceptions known to the author are certain cases where cells near the edge of skin transplants have appeared to develop nonautonomous pigment which must have been determined in some way by a very limited type of migration of pigment-forming cells between the marginal cells of host and donor tissues (REED and HENDERSON 1940).

The final alternative possibility that conditions in the haematopoietic tissues and the hair follicle both independently produce a situation in which the *W*-genes have similar or identical effects must be considered seriously. To prove local gene action is extremely difficult, to disprove it somewhat easier, and the author feels that the best procedure is to test out all possibilities of action from a distance. Possibly in favor of local action, however, is the observed exactness in relation between gene dosage and final character in both tissues. In his analysis of the nature of the action of the *ff* gene-pair (siderocyte anemia, tail flexure, belly spot) GRÜNEBERG (1942b) makes considerable use of the fact that the anemia is a much more regular manifestation of the gene than is tail flexure as evidence for his conclusion that the anemia is the primary result of gene action. It would be impossible to say in the case of the *W*-series which effect is most regular, as both blood and pigment effects occur in all cases in exact concordance with the genotype. Either the dependence of the degree of pigmentation upon the anemia is in very delicate adjustment, or precursors of both tissues must be identically affected by some externally-located effect of the gene during embryological development, or both phenomena must result from independent original gene action in the affected tissues.

Further mention might be made at this point of the nature of the $W^v w$-ww pigment reduction. It appears to be a general change in the degree or level of pigmentation, affecting all qualitatively different types of pigment which have been tested. Two other examples of such general change have been established (RUSSELL 1949b). The first is the change produced by *C*-series (albino) gene substitutions in which the gene action appears to result in a change in the concentration of the enzyme dopa-oxydase (RUSSELL, L. B., and W. L. RUSSELL 1948). The second is the non-genic change in pigmentation level along the axis of the hair in many genotypes with an intermediate amount of total pigmentation. Because of the close parallelism of effect of $W^v w$-ww substitution on blood and pigmentation this knowledge of the nature of the pigment effect may be of help in interpreting the nature of the basic gene action on blood as well as on hair pigment-producing cells.

Although the $W^v w$ pigment reduction is allied to the albino-series reduction,

it is not identical with it, since the two allelic series have somewhat different types of effects upon medullary granule number. An important point in the interpretation of the effect of $c^{ch}c^{ch}$-CC substitution (RUSSELL 1949b) on a black or brown background was that granule number is not affected, and any reduction in total pigment is due to a reduction in granule size. The assumption made was that all available sites of pigment deposition were already active at the chinchilla level, and addition of further enzyme could act only by increasing the size of particles at each site. The fact that the medullary granule numbers in full color blacks (total from the 10th to the 50th fields, 451), full color browns (total, 434), chinchilla blacks (total, 433), and chinchilla browns (total, 418) are almost identical, while the estimated total volume of medullary pigment varies widely, with full color blacks very high (783 units), full color browns low (318 units), chinchilla blacks intermediate (400 units) and chinchilla browns low (303 units) (RUSSELL 1948), strongly supports the concept that in all of these a maximum number of granules has been reached. If $W^v w$ reduction were identical in nature to C-series reduction, its effect on full color blacks certainly should have been a reduction in granule size much greater than any effect on number. Actually, in this case number is much more effected than is size (figure 2, tables 4 and 7). In the two types where size is affected, full color and chinchilla black, the reduction is significant but very small. The reduction in granule number is much greater. A curious and probably significant fact is that in each of three backgrounds where *ww* genotypes have identical medullary granule numbers but differing total volumes of pigment, $W^v w$ substitution has the same proportional effect on granule number (in full color black, 68 percent, in chinchilla black, 74 percent, and in full color brown, 75 percent), and of course very different proportional effect on pigment volume. Two other backgrounds, where the *ww* type was not near this maximum, had very different proportional reductions in granule number (full color yellow, 86 percent, and pink-eyed sepia, 47 percent). This suggests the possibility that the $W^v w$ action on pigment intensity either removes or incapacitates some of the sites of pigment deposition, and the amount of reduction in any particular background depends upon the relation of the pigment producing potentialities of the rest of the genotype to the new restricted limit on sites of pigment deposition.

Thus this paper has summarized evidence on the nature of dominance relations of W and W^v in their effect upon erythrocytes and on hair pigment intensity, with the conclusion that although the W gene has no dominance in either tissue, W^v is dominant in both, a single dose producing a significant effect. Emphasis has been placed on the close parallelism of the effects of the entire W-series of genes upon the blood and pigment-producing tissues, especially in these peculiar dominance relationships. The fact has been stressed that in both cases the gene W with no effect in one dose has a severe effect in two doses (WW), while the gene W^v with some effect in one dose has a milder effect (compared to WW) in two doses ($W^v W^v$). An attempt has been made to analyze the nature of the W^v dominant effect on hair pigmentation, with the conclusion that it is a general change in all pigmentation, probably in-

volving restriction in sites of pigment deposition. Suggestions have been made as to the possible significance of these results to a further analysis of the pleiotropic effects of the *W*-series genes.

SUMMARY

1. Studies were made of the blood picture of month-old littermates in stocks approaching genetic uniformity. In litters segregating for $W^v w$ and *ww*, the $W^v w$ individuals had significantly lower erythrocyte counts and hematocrit readings, significantly higher mean cell volumes than their *ww* littermates. In litters segregating for *Ww* and *ww* there were no significant differences between the genotypes.

2. Studies were made of the number and size of hair pigment granules in five differently colored $W^v w$ genotypes. In full-color blacks, chinchilla blacks, full-color browns, pink-eyed sepia, and full-color yellow backgrounds, the $W^v w$ type has fewer pigment granules than its *ww* counterpart. In some types there is also reduction in granule size.

3. An analysis of variance of medullary granule numbers in litters segregating for $A^v aBBCCDDPPW^v w$-*ww* (the least reduced of the five stocks mentioned above) proved statistically that the difference between the $W^v w$ and *ww* genotypes is highly significant and is by far the greatest source of variability.

4. Studies were made of the number and size of pigment granules in litters segregating for *Ww* and *ww* in a full-color black stock (*aaBBCCDDPPWw-ww*). There is no reduction in granule size, and an analysis of variance of medullary granule number proved statistically that there is no significant difference between *Ww* and *ww* segregants.

5. The observed effects of $W^v w$ substitution upon granule number and size are compatible with the concept that this substitution causes a reduction in the general level of pigmentation.

6. In both blood and pigment-forming cells of the hair, *W* has no effect in one dose, a very severe effect in two doses, while W^v has a significant effect in one dose and a milder effect than *W* in two doses. This close correlation in the dominance relations of the two alleles indicates a very close connection between the *W*-gene action leading to blood changes and that leading to pigment-intensity changes.

7. The bearing of these findings upon further analyses of the pleiotropic effects of the *W*-series and determination of the nature of gene action is discussed.

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THE EFFECT OF INBREEDING UPON GROWTH IN JERSEY CATTLE[1]

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INTRODUCTION

EAST and JONES (1919) and CASTLE (1930) reviewed the significant experiments with plants and laboratory animals that contributed to the formulation of the modern viewpoints concerning the effects of inbreeding. The phenomena associated with inbreeding: decline in vigor, increased uniformity within the inbred stock, increased prepotency in outside crosses, isolation of abnormal types, etc., were shown to be consequences of the increase in the proportion of homozygous gene pairs.

While the inbreeding results obtained with laboratory animals are believed by geneticists to apply to cattle in a general way, such beliefs, representing, as they do, an extrapolation to relatively unknown genetic material, must of necessity be tentative until a sufficient number of adequately controlled experiments dealing with the effects of inbreeding upon cattle have been reported.

The literature dealing with inbreeding experiments with cattle covers only the last two decades and is not extensive. This situation is understandable since perfectly regular inbreeding systems are not feasible for cattle. Furthermore, economic considerations and the relatively great time intervals between generations (four to five years) hamper the design of adequately controlled experiments. These obstacles have been materially reduced within the last three decades by means of improved statistical techniques and by WRIGHT'S (1922) publication of a method of calculating the coefficient of inbreeding which is strictly proportional to the probable loss in heterozygosis as a result of inbreeding alone. This inbreeding coefficient can be calculated for pedigrees exhibiting any degree of irregularity.

In general, the reported experiments dealing with the effect of inbreeding upon various size characteristics in dairy cattle have suggested that inbreeding exerts a depressing affect upon these characteristics.

WOODWARD and GRAVES (1933) found that intense inbreeding of grade Guernsey and grade Holstein-Friesian cattle caused a decrease in birthweight, a retardation in the growth rate of calves, and a reduction in mature weight. However, the reduction in mature weight was not proportionally as great as

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that for birthweight. The birthweights of calves and the weights of mature cows were not depressed so markedly in the inbred Holsteins as they were in the inbred Guernseys. They emphasized that the nature of the results depended greatly on the genetic composition of the sires chosen.

In their recent study of grade Holsteins, WOODWARD and GRAVES (1946) found that inbreeding greatly depressed birthweights, retarded growth, increased mortality, and reduced the size (weight), vigor, and production of the cows. The harmful effects became apparent when the coefficient of inbreeding exceeded 25 percent. Birthweights of calves declined from an average of 81.5 pounds for outcrossed calves to 65.9 pounds for inbreds with coefficients of inbreeding over 50 percent. Average weights for inbred and outcrossed heifers were compared for ages 3, 6, 18, and 24 months. At each age the outcrosses were heavier than the inbreds. However, the differences between the two groups decreased with age from birth to 24 months.

BARTLETT, REECE, and MIXNER (1939) studied the effect of inbreeding on height at withers, body-weight, and heart girth of Holstein-Friesian cattle. A comparison of the average size of 60 inbred heifers, with coefficients of inbreeding ranging from 5 percent to over 15 percent, with that of outcrossed heifers, at birth and at 5 and 10 months of age, indicated no signifiant difference between the two groups. BARTLETT, REECE, and LEPARD (1942) reporting on a continuation of the above study compared inbred and outbred heifers for size at birth and rate of growth through 22 months of age. They found no relation between the characteristics studied and the intensity of inbreeding.

BARTLETT and MARGOLIN (1944) studied height at withers, body weight, and heart girth of 67 outbred and 76 inbred Holstein heifers with coefficients of inbreeding ranging from 5 percent to over 20 percent. They concluded that Holstein-Friesian cattle could be inbred without decreasing weight or size, provided the inbreeding did not rise above 20 percent. Females more highly inbred than this developed normally to about the first calving, but then their development became abnormal. The findings of MARGOLIN and BARTLETT (1945) were in agreement with their earlier results.

DICKERSON (1940), in a preliminary analysis of the data from three Holstein herds, found that the birthweights of calves of both sexes with an average inbreeding coefficient of 16 percent averaged nearly 10 percent lighter than those of outcrossed calves by the same sires. A tentative suggestion was made that the size difference in favor of the outbreds became proportionally smaller with growth up to six months of age.

NELSON (1943) studied the effects of inbreeding on body weight and several body measurements in a closed Holstein herd at IOWA STATE COLLEGE. His results suggested that inbreeding decreased growth rates at ages under four years, but that the inbreds tended to grow faster than the outcrossed at some time after 3 years of age. Mature size seemed unaffected, but the data on mature animals were few. Inbreeding in the herd averaged 5 percent, but for individuals ranged as high as 28 percent.

BAKER, MEAD, and REGAN (1945) analyzed growth data on height at withers, body weight, and heart girth of 88 daughters of one sire in the inbred

Holstein herd at the UNIVERSITY OF CALIFORNIA. Coefficients of inbreeding ranged from zero percent to about 42 percent and the period of growth studied was from 6 months to 45 months of age. They found that inbreeding caused a significant decrease in size for each of the three characteristics studied.

The present study deals with the effect of inbreeding upon growth in Jersey cattle. Data on the three characteristics—height at withers, heart girth (i.e., chest girth), and body weight—are analyzed.

SOURCE OF DATA

The data used in this study were obtained from Jersey females in the UNIVERSITY OF CALIFORNIA dairy-cattle-breeding experiment, and were collected by PROFESSORS S. W. MEAD and W. M. REGAN over a 24 year period, from 1921 to 1944. MEAD, GREGORY, and REGAN (1946) described the background of the experiment, and REGAN, MEAD, and GREGORY (1947) discussed the care, management, and other known environmental factors that might have affected the growth and vitality of the stocks.

Sire-daughter matings were employed whenever possible; but since this type of mating can be only temporary, there were many irregularities in the mating systems used.

The original plan called for the development of three inbred lines through sire-daughter matings, with each sire to be followed by a progeny tested inbred son descended from a succession of high producing females. Sires were to be selected for their ability to transmit high milk yield. During the 1930's one line was discontinued because of a highly variable milk yield and the two remaining lines were merged due to a curtailment of funds and an attempt to eliminate two sires each of which was found to be heterozygous for a different recessive lethal gene (MEAD, GREGORY, and REGAN 1946).

Within sire groups there was no culling of female progeny unless a heifer failed to breed after repeated matings.

The progeny of one sire included dwarfs. These dwarfs were not included in this study since MEAD, GREGORY, and REGAN (1946) demonstrated that the sire in question was heterozygous for a recessive gene conditioning dwarfness.

The total number of animals included in this study is 322. They were sired by 15 bulls. The number involved at a given age ranges from 274 at birth to 63 at 56 months, an age attained during the third lactation period. The variation in the number of animals involved at different ages is due to the following causes:

(1) Not all animals had weights or measurements recorded for each age studied.

(2) For some of the ages studied there was no variation in the inbreeding coefficients of some sires' progeny for which weights and measurements were available (the effect of inbreeding was analyzed on an intra-sire basis for height and on an intra-sire, intra-year basis for heart girth and weight respectively).

(3) In studying heart girth and weight during lactation, only animals that

were of similar age and in the same stage of the same lactation (that is, first, second, or third lactation) were used.

Coefficients of inbreeding (WRIGHT 1922) were calculated to measure the intensity of inbreeding. The average intensity of inbreeding was 17 percent for animals with recorded birthweights and ranged from 15 percent to 10 percent for animals involved at other ages studied. Individual inbreeding coefficients ranged as high as 47 percent.

ANALYSIS OF DATA

The data for height cover a period from 1924 to 1944, during which time the herd was essentially a two sire herd. Altogether, 11 sires were used during the period in question. Several of the sires were in service for periods extending over many years. Since the percent of inbreeding tended to increase with years, the extended period of service of some sires posed the question of a possible confounding of a time trend with inbreeding effects. An inspection of intra-sire scatter diagrams of height plotted against year of birth indicated no systematic variation of height with year of birth except for the progeny of two of the sires, 320A and 300D, both of which were in service concurrently during the period from 1924 to 1931. The remaining sires were in service at some time during the period from 1930 to 1944.

For the two sires in question, 320A and 300D, the scatter diagrams indicated a marked positive linear regression of height on year of birth. An intra-sire partial regression analysis of height on year of birth with percent of inbreeding held constant revealed a statistically significant linear increase of height on year of birth for the ages 12 months, 24 months, and 36 months respectively. For the sires used subsequent to 1930 a similar analysis revealed no statistically significant results.

In view of the above preliminary investigation it was decided to analyze the effect of inbreeding upon height on an intra-sire basis after correcting the heights of the progeny of 320A and 300D for the time trend. The following corrections for year of birth were estimated from the aforementioned regression analysis:

CHARACTERISTIC	CORRECTION FOR YEAR OF BIRTH (CM PER YEAR)
6th Month Height	0.4
12th Month Height 24th Month Height 36th Month Height 54th Month Height	0.6

Intra-sire scatter diagrams of weight and heart girth, respectively, plotted against year of birth indicated in general a positive regression of these charac-

teristics on year of birth. However, this effect was so varied from sire to sire, both as to the magnitude of a linear component of regression and also according to the possible significance of higher degree terms in the regression, that it was decided to sidestep the whole question of a time trend in so far as an intra-sire, intra-year analysis would permit.

Linearity of Regression of Height, Weight, and Heart Girth, Respectively, on Percent of Inbreeding

Although scatter diagrams suggested that the regressions of height, weight, and heart girth, respectively, on percent of inbreeding were linear, it was thought advisable to test this assumption statistically in as much as the range of inbreeding within some groups was as great as 40 percent. Since the data were analyzed on an intra-sire basis for height and on an intra-sire, intra-year basis for weight and heart girth, they were not too suitable for investigating the linearity of regression of a trait on the percent of inbreeding in that within many groups the range of inbreeding was slight, or only two levels of inbreeding were present. It was decided to make such an investigation employing those groups in which the range of inbreeding was at least 25 percent, and in which more than two levels of inbreeding were present.

For weight and heart girth at 12 months of age respectively (the age at which most data were available) the ranges of inbreeding for the groups used were:

SIRE (INTRA-SIRE) (INTRA-YEAR)	NUMBER OF ANIMALS WITHIN THE GROUP	RANGE OF INBREEDING IN PERCENT
300D	6	0–40
320A	5	0–25
333C	9	3–38
333C	5	3–44
300C	4	0–26
386A	6	0–36
428A	4	0–38
433A	9	0–28
TOTAL NUMBER OF ANIMALS	57	

For sixth month height (the age at which the estimated inbreeding effect was maximum) the data available were as shown in table at top of facing page.

A second degree term in the regression of each of the traits on percent of inbreeding was tested for significance.

$$E(Y_{ij}) = a_j + b_j X_{ij} + c_j X_{ij}^2$$

Y_{ij} = the observed value of the trait for the ith animal in the jth group.

$E(Y_{ij})$ = the expected value of Y_{ij}

X_{ij} = the percent of inbreeding of animal ij.

SIRE (INTRA-SIRE)	NUMBER OF ANIMALS WITHIN THE GROUP	RANGE OF INBREEDING IN PERCENT
300D	39	0–40
320A	28	0–35
433A	15	0–41
386A	13	0–36
333C	9	8–47
428A	4	0–38
TOTAL NUMBER OF ANIMALS	108	

Hypothesis tested: $c_j = 0$, for all j.

The test results presented in table 1 support an hypothesis of linearity of

TABLE 1

Tests of significance of departure from intra-group linear regressions of height, weight, and heart girth respectively on percent of inbreeding.

TRAIT	SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F	$F_{.05}$	$F_{.01}$
6th Mo. Height	Deviations from mean	102	2,173.00				
	Deviations from linear regression	96	1,678.10	17.48			
	Linear Regression	6	494.90	82.48**	4.72		2.99
	Deviations from curved regression	90	1,560.90	17.34			
	Curvilinearity of regression	6	117.20	19.53	1.13	2.20	
12th Mo. Weight	Deviations from mean	48	85,257.90				
	Deviations from linear regression	39	51,054.74	1,309.10			
	Linear Regression	9	34,203.16	3,800.35**	2.90		2.89
	Deviations from curved regression	30	48,068.53	1,602.28			
	Curvilinearity of regression	9	2,986.21	331.80	0.21	2.21	
12th Mo. Heart Girth	Deviations from mean	48	817.08				
	Deviations from linear regression	39	476.82	12.23			
	Linear Regression	9	340.26	37.81**	3.09		2.89
	Deviations from curved regression	30	409.78	13.66			
	Curvilinearity of regression	9	67.04	7.45	0.55	2.21	

** See p. 731.

TABLE 2

Intra-sire regression of height on percent of inbreeding.

GROUP	NO. OF ANIMALS	NO. OF SIRES	AVERAGE PERCENT OF INBREEDING	REGRESSION COEFFICIENT (b): HEIGHT ON PERCENT OF INBREEDING						
				1ST MO.	3RD MO.	6TH MO.	12TH MO.	24TH MO.	36TH MO.	54TH MO.
1	82	8	11.8	−0.095**	−0.121**	−0.179***	−0.162***	—	—	—
2	163	11	14.3	—	—	−0.147***	−0.126***	—	—	—
3	195	9	14.9	—	—	—	−0.108***	−0.054**	—	—
4	168	9	14.2	—	—	—	−0.097***	−0.037	−0.026	—
5	117	9	12.1	—	—	—	−0.098**	−0.036	−0.019	−0.007
Average Height (in cm)				67.3	77.5	94.1	109.7	122.4	126.0	127.4
Coefficient of Variation (in percent)				5.2	4.2	4.0	2.7	2.1	2.3	2.0

,* See p. 731.

regression of height, weight, and heart girth respectively on percent of inbreeding for the range of inbreeding dealt with in these data.

The Relation of Height to Inbreeding

Intra-sire regressions of height on percent of inbreeding (b) were calculated for various ages from 1 month to 54 months. These are presented in table 2. One asterisk (*), two asterisks (**), and three asterisks (***) indicate that the estimated parameter is significantly different from zero at the 5 percent, 1 percent, and 0.1 percent levels of probability, respectively. In table 2, Group 1 refers to the total number of animals each of which had recorded measurements for 1, 3, 6, and 12 months of age. The total number of animals each of which had recorded measurements for 6 and 12 months of age is designated as Group 2. The remaining groups are similarly defined.

In table 3 there are presented intra-sire regressions of "terminal" height on percent of inbreeding, independent of "initial" height, for various growth periods. These partial regression coefficients give some indication of the nature of the inbreeding effect manifested at a given age ("terminal") which is unaccounted for by the effect indicated at an earlier age ("initial").

Expressing the regression coefficient as a percentage of the corresponding mean height is possibly a more practical means of observing the relation of the effect of inbreeding to age. It also serves as a basis for comparing the relative effect of inbreeding upon height, weight, and heart girth. The regression coefficients as percentages of corresponding mean heights are presented in table 4.

The regression of height on percent of inbreeding is negative for all the ages considered (table 2). Its absolute value, that is, disregarding negative signs, reaches a maximum at 6 months of age and then decreases steadily thereafter. For ages up to and including 24 months these coefficients are significant; some at the 0.1 percent and others at the 1 percent level. An interpretation of these coefficients is that the deleterious effect of inbreeding is directly proportional to the absolute size of the coefficients with negative signs. Making such an interpretation, it would seem that the inbred animals were lower at the withers at 1 month of age and grew more slowly up to some age between 6 and 12 months than did the outcrossed animals, but from such an age on, the inbreds began to catch up with the outcrossed so that at 54 months of age the effect of inbreeding appeared negligible. Expressing these regressions as percentages of corresponding mean heights indicates that at 6 months of age for each percent of inbreeding there resulted on the average a decrease of 0.16 percent in mean height while at 54 months of age, the corresponding decrease was 0.01 percent.

The effect of inbreeding on rate of growth is probably most clearly presented in table 3 in which the partial regressions are the intra-sire regressions of "terminal" height on percent of inbreeding, independent of "initial" height, for various growth periods. For the animals in group 1, a coefficient of -0.113^{**} cm for the period 1 to 6 months of age indicates that the inbred animals were growing more slowly than the outcrossed during this period. Making a similar interpretation for other periods, it is seen that for the sixth to twelfth month,

TABLE 3

Intra sire regression of "terminal" height on percent of inbreeding independent of "initial" height for various growth periods.

GROUP†	PARTIAL REGRESSION COEFFICIENT				
	1ST MO. TO 6TH MO.	6TH MO. TO 12TH MO.	12TH MO. TO 24TH MO.	12TH MO. TO 36TH MO.	12TH MO. TO 54TH MO.
1	−0.113**	−0.068**	—	—	—
2	—	−0.036	—	—	—
3	—	—	0.012	—	—
4	—	—	0.019	0.033	—
5	—	—	0.019	0.045	0.047

† The same groups as listed in Table 2.
** See p. 731.

the difference between the growth rates of inbreds and of outcrosses has decreased. The positive regression coefficients for periods beyond 12 months of age, increasing in size to 0.047 cm for the period 12 to 54 months of age, indicate that the inbred animals tended to grow faster than the outcrossed after some age between 6 and 12 months. Although only two of these coefficients are statistically significant, the striking regularity of their variation with age lends added import to them when taken as a whole.

TABLE 4

Regression coefficients of height on percent of inbreeding presented as percentages of corresponding mean heights.

GROUP†	AGE IN MONTHS						
	1	3	6	12	24	36	54
1	−0.14	−0.16	−0.19	−0.15	—	—	—
2	—	—	−0.16	−0.11	—	—	—
3	—	—	—	−0.10	−0.04	—	—
4	—	—	—	−0.09	−0.03	−0.02	—
5	—	—	—	−0.09	−0.03	−0.02	−0.01

† The same groups as listed in Table 2.

The Relation of Heart Girth and Weight to Inbreeding

In estimating the effect of inbreeding upon heart girth and weight respectively, data on animals for ages prior to breeding and during their first, second, and third lactation periods were analyzed. Pregnancy and lactation as significant sources of variation in these characteristics were taken into consideration.

For each lactation, the range from four to six months after calving was considered a period in which the effects of parturition, lactation, and the succeeding gestation, as causes of variation in weight, and heart girth would be minimized. In no case did the period within which measurements were utilized in

the analysis extend beyond the fourth month of a succeeding gestation. PUTNAM and HENDERSON (1946) and MORGAN and DAVIS (1936) in their studies of the effect of pregnancy and parturition on the weights of dairy cows, found that in the second and successive gestations gain in weight over and above normal weights was not great before the fifth month of pregnancy.

The number and ages of animals for which data were available for the first lactation period are as follows:

NUMBER OF ANIMALS	NUMBER OF INTRA-SIRE INTRA-YEAR GROUPS	AGE IN MONTHS OF ANIMALS WITHIN GROUPS
33	8	28
10	2	29
15	4	31
14	4	32
6	1	33
Total Number of Animals: 78	Total Number of Groups: 19	Average age in months: 30

The number of animals in the second lactation group was 83 with an average age of 43 months. The range of ages within sire-year groups averaged six months. The number of animals in the third lactation group was 63 with an average age of 56 months. The range of ages within sire-year groups averaged 8 months. For the second and third lactation groups respectively observed weights were corrected for month of age. A linear regression of weight on month of age was assumed a close enough approximation to the corresponding segment of the true growth curve for the intra-group age ranges encountered. For each lactation period the estimated correction for age was three pounds per month. Observed heart girths were not adjusted in as much as they were recorded to the nearest centimeter and the estimated correction was of the order of 0.1 cm per month of age.

Heart Girth

The intra-sire intra-year regressions of heart girth on percent of inbreeding (b) for different ages are shown in table 5. In the column headed (b/m) percent, these regressions are expressed as percentages of corresponding mean heart girths.

The regressions of heart girth on percent of inbreeding are all negative, but decrease in absolute magnitude with advancing age after six months of age. This is reflected in the variation of the statistic (b/m) percent with respect to age, which would seem to indicate that the average heart girth of inbred animals, although smaller than that of the outcrossed, tended to approach the latter during the period of growth subsequent to six months of age.

Weight

Birth weights were adjusted for the age of the dam since an analysis of the

TABLE 5

Intra-sire, intra-year regressions of heart girth on percent of inbreeding for different ages.

GROUP	NUMBER OF ANIMALS	NUMBER OF INTRA-SIRE INTRA-YEAR GROUPS	AGE IN MONTHS	AVERAGE PERCENT OF INBREEDING	AVERAGE HEART GIRTH IN CM m	REGRESSION OF HEART GIRTH ON PERCENT OF INBREEDING b	$\left(\frac{b}{m}\right)\%$	COEFFICIENT OF VARIATION (IN PERCENT)
1	66	12	1	13.4	65.7	−0.120*	−0.18	7.2
1	66	12	6	13.4	105.7	−0.231**	−0.22	6.1
1	66	12	12	13.4	135.2	−0.227***	−0.17	3.6
—	127	20	6	14.1	108.3	−0.167*	−0.15	6.2
—	153	23	12	14.6	138.3	−0.163***	−0.12	3.5
1st Lactation	78	19	30	15.3	169.9	−0.074	−0.04	3.4
2nd Lactation	83	19	43	15.2	170.9	−0.045	−0.03	3.3
3rd Lactation	63	15	56	10.6	173.0	−0.011	−0.01	2.8

*,**,*** See p. 731.

data indicated that first calves averaged two pounds less than calves from subsequent calvings.

The intra-sire, intra-year regressions of weight on percent of inbreeding (b) for different ages are shown in table 6. The regressions are all negative, but,

TABLE 6

Intra-sire, intra-year regressions of weight on percent of inbreeding for different ages.

GROUP	NUMBER OF ANIMALS	NUMBER OF INTRA-SIRE INTRA-YEAR GROUPS	AGE IN MONTHS	AVERAGE PERCENT OF INBREEDING	AVERAGE WEIGHT IN POUNDS m	REGRESSION OF WEIGHT ON PERCENT OF INBREEDING b	$\left(\frac{b}{m}\right)\%$	COEFFICIENT OF VARIATION (IN PERCENT)
—	274	38	Birth	17.0	55.4	−0.154***	−0.28	12.3
—	127	20	6	14.1	279.6	−1.305**	−0.47	14.7
—	153	24	12	14.7	527.6	−1.797***	−0.34	9.2
1st Lactation	78	19	30	15.3	923.9	−2.137	−0.23	8.9
2nd Lactation	83	19	43	15.2	929.1	−2.644*	−0.28	9.9
3rd Lactation	63	15	56	10.6	980.4	−1.001	−0.10	8.8

*,**,*** See p. 731.

unlike the cases for height and heart girth, the coefficients increase in absolute size through the second lactation period (43 months of age). However, if the regressions are expressed as percentages of corresponding means (b/m) percent, they reach a maximum absolute size at six months of age and then tend to decrease thereafter. This pattern of variation with age is similar to that found for height and heart girth. It was decided to use the statistic (b/m) percent rather than the regression coefficient for making comparisons between the relation of the inbreeding effect to age for the three characteristics, height, weight, and heart girth. This statistic seemed to lend itself to interpretation from a practical point of view and to be more compatible with the exponential

nature of the growth curves for height, weight, and heart girth of dairy cattle (BRODY 1927).

DISCUSSION

The Effect of Inbreeding Upon Size

The regression coefficients of each of the characteristics, height, weight, and heart girth, on percent of inbreeding are negative for all ages considered (tables 2, 5, and 6). This suggests that in general inbreeding caused a decrease in size. For the ages considered up through 12 months, all coefficients were statistically significant; five at the 0.1 percent probability level, three at the 1 percent level, and one at the 5 percent level. For ages beyond 12 months two out of nine coefficients were significant. This rather general lack of significance of coefficients for ages beyond 12 months appears to be due to the relatively fewer data available and to the fact that the regression coefficients for height and heart girth and the regressions expressed as percentages of the corresponding means for weight tended to decrease in absolute magnitude for ages subsequent to six months.

Inasmuch as individual coefficients of inbreeding ranged as high as 47 percent, the possibility of a non-linear regression of the characteristics studied on percent of inbreeding was investigated. A second degree term in the regression of sixth month height, twelfth month weight, and twelfth month heart girth respectively on percent of inbreeding was tested for significance. The results presented in table 1 support an assumption of linearity of regression in each case. However, the data analyzed were few. This point has not been investigated in the reported experiments dealing with dairy cattle.

With the exception of some limited observations on Guernseys (WOODWARD and GRAVES 1933), the literature dealing with the effects of inbreeding upon size of dairy cattle concerns Holsteins. It is of interest to compare the results obtained for Jerseys with those reported for Holsteins.

The significant decrease found in birthweights due to inbreeding (table 6) is in agreement with the findings of WOODWARD and GRAVES (1933, 1946), DICKERSON (1940), and NELSON (1943). The New Jersey station found no effect of inbreeding upon birthweights, however, their data were few and the average level of inbreeding was low.

WOODWARD and GRAVES (1933, 1946) found that inbreeding caused a decrease in weight at 3, 6, 18, and 24 months of age and at maturity; but the difference in favor of the outcrossed animals became proportionally smaller with advancing age. NELSON (1943), in studying weight and several body measurements including height and heart girth, found evidence that inbreeding decreased growth rate at ages under four years, but mature size seemed unaffected. He further noted a tendency for inbreds to grow faster than non-inbreds at some time after three years of age. The results of both of these studies are in general agreement with those of the present study for ages subsequent to 6 months. On the other hand, BAKER MEAD, and REGAN (1945) found that inbreeding caused a significant decrease in weight, height, and heart girth which was proportionally equal throughout the period studied from 6

months to 45 months of age. However, in statistically estimating the effect of inbreeding, the measurements on inbred animals involved were just for ages falling within the range of 18 to 33 months. The data for certain levels of inbreeding were meager for ages outside of the indicated range. BARTLETT and MARGOLIN (1944) found that inbreeding had no effect upon size until after the first calving.

The Effect of Inbreeding Upon Rate of Growth

In tables 4, 5, and 6 the effect of inbreeding on height, heart girth, and weight respectively is expressed by the statistic (b/m) percent, where b is the regression coefficient of the characteristic on the percent of inbreeding and m is the corresponding mean value. The pattern of variation of this statistic with age, and the regressions of "terminal" height on the percent of inbreeding, independent of "initial" height, for various growth periods (table 3) seemed to warrant the following interpretation in terms of growth rates: the inbred animals were smaller at birth and grew more slowly up to about the sixth month of age than the outcrossed animals, but from some time between the sixth and the twelfth months of age the inbreds began to grow more rapidly than the outcrossed and continued to do so for the remainder of the period studied.

WOODWARD and GRAVES (1946) and DICKERSON (1940) suggested that the difference in weight in favor of the outbred over the inbred animals was proportionally greater at birth than at six months of age. This is at variance with the results of this study. The findings of REGAN, MEAD, and GREGORY (1947) suggest the possibility that the difference in results may be due to a breed difference in the effect of inbreeding on vigor prior to six months of age. They found in their study of the relation of inbreeding to calf mortality that Jersey calves were less viable than Holstein calves, and that the increase in the percentage of mortality that accompanied inbreeding was greater for Jerseys than for Holsteins. Their findings apply to the mortality up to four months of age of single calves born alive.

SUMMARY

Data collected over a period of 24 years, from 1921 to 1944, on body weight, height at withers, and heart girth (i.e. chest girth) of females in the UNIVERSITY OF CALIFORNIA Jersey herd have been analyzed. Records on 322 animals were studied in an investigation of the effect of inbreeding upon growth during the period from birth to 56 months of age. Coefficients of inbreeding averaged 15 percent and for individuals ranged from zero percent to 47 percent.

A covariance analysis of height, weight, and heart girth, respectively, and the percent of inbreeding indicated that inbreeding caused a decrease in each of these characteristics. Statistical tests supported an assumption of linearity of regression of each characteristic on percent of inbreeding.

The magnitude of the inbreeding effect varied with age and characteristic. Weight was most affected. The variation with age was the same for each characteristic, with the maximum effect occurring at six months of age. At six months of age, an increase of one percent in inbreeding caused a decrease of

0.47 percent in mean weight, 0.15 percent in mean heart girth, and 0.16 percent in mean height. The corresponding figures at $4\frac{1}{2}$ years of age were a decrease of 0.10 percent in mean weight, 0.01 percent in mean heart girth, and 0.01 percent in mean height. For birthweight the corresponding decrease was 0.28 percent.

Inbreeding appeared to affect the prenatal and post natal rate of growth. The inbred animals were smaller at birth and grew more slowly up to about the sixth month of age than the outcrossed animals, but at some time between the sixth and twelfth month of age the inbreds began to grow more rapidly than the out-crossed and continued to do so for the remainder of the period studied.

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GENETICS

A PERIODICAL RECORD OF INVESTIGATIONS BEARING ON HEREDITY AND VARIATION

Founded in 1916

by

GEORGE H. SHULL

with the cooperation of

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CORRIGENDA

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Page 550, line 7, for "let $\omega^{(0)}(x; y)$ be . . . "
read "Let $w^{(0)}(x; y)$ be . . . "

Page 550, line 9, for "$w^{(0)}(x; y) = w^{(0)}(y; x)$"
read "$\omega^{(0)}(x; y) = \omega^{(0)}(y; x)$."

Volume 34, 1949

Page 403, last paragraph, line 2, for "phenotype" read "genotype."

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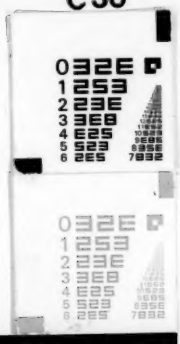
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